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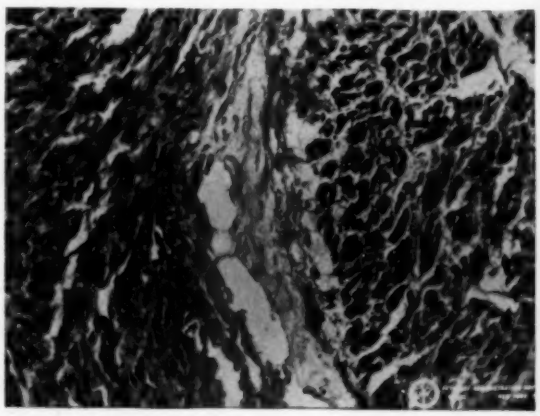

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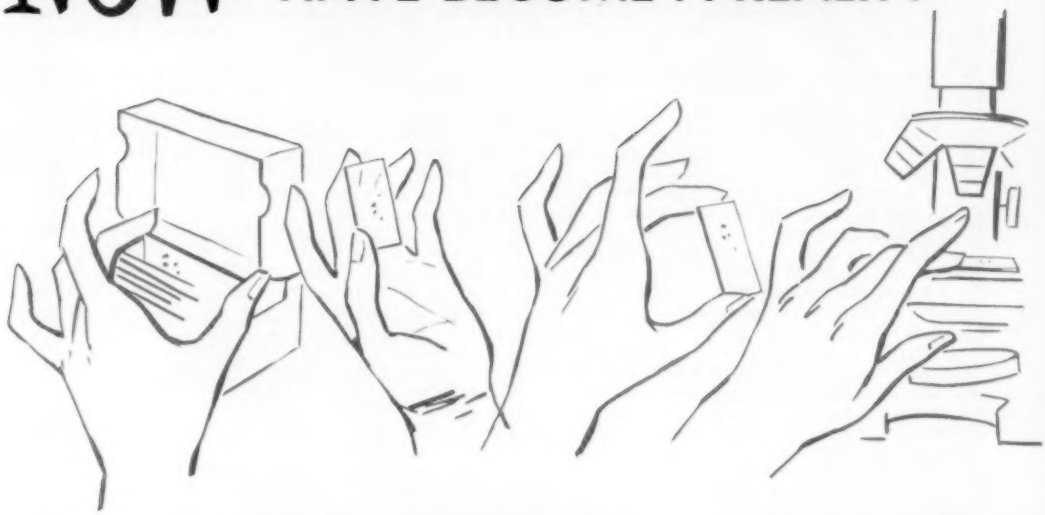

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A Histochemical Comparison of Primary Thyroid Hyperplasia and Adenomatous Goiter

HAROLD L. HALEY, M.D.
GRACE M. DEWS, B.A.
and
SHELDON C. SOMMERS, M.D., Boston

Thyroid glands with primary hyperplasia and adenomatous goiter are ordinarily distinguished pathologically by characteristic architectural changes. Abnormalities of thyroid metabolism as reflected by physiologic and biochemical tests also have been extensively studied. Changes in human thyroid function at the cellular level are less well understood. For this reason it appeared worth while to investigate, with a battery of histochemical tests, surgical material from the two commonest human thyroid diseases and normal controls. Discrimination by histochemical means between primary thyroid hyperplasia and nodular goiter might prove useful in classifying early or atypical examples of these diseases.

MATERIALS AND METHODS

Twelve surgical patients with primary hyperthyroidism were studied. They had routine preoperative preparation with propylthiouracil and strong iodine solution U. S. P. Eighteen cases of adenomatous goiter (nontoxic nodular goiter) also were examined. From autopsies of eight persons without clinical or pathologic evidences of any thyroid disease, normal control thyroid tissue was obtained.

From the Cancer Research Institute, New England Deaconess Hospital; Department of Surgery, Peter Bent Brigham Hospital, and Laboratory of Pathology, Massachusetts Memorial Hospitals.

This work was done under U. S. Atomic Energy Commission Contract AT (30-1)-901 with the New England Deaconess Hospital.

Specimens were prepared by freezing-drying, with a method and apparatus described elsewhere.[12] Hematoxylin and eosin stains were made from paraffin sections of all frozen-dried blocks. Additional thyroid specimens from the same cases were routinely fixed in Zenker's solution, Autotechnicon processed, and paraffin sections made and stained with hematoxylin and eosin for pathologic diagnosis. These slides were available for comparison and as reference.

Histochemical procedures employed included

(1) Methyl green pyronine stain (modified) [3]
(2) Periodic acid-Schiff reaction (McManus) [14]
(3) Sulfhydryl stain (Barrnett and Seligman) [1]
(4) Benzidine peroxidase stain (Gomori, modified) [10]
(5) Potassium stain (MacCallum) [13]
(6) Alkaline phosphatase stain (Gomori) [7]
(7) Acid phosphatase stain (Gomori) [7]

In examining the histochemical preparations the four major thyroid cell types identified by Goormaghtigh and Thomas,[11] and used in histophysiologic studies by Goddard and Sommers,[9] were considered:

Type	Shape	Inferred Function
I	Cuboidal	Slowly secreting colloid into follicle
II	Columnar	Actively resorbing colloid from follicle
III	Trapezoidal	Actively secreting colloid into follicle
IV	Endothelioid	Resting, or slowly secreting

In primary thyroid hyperplasia, Type II resorptive cells are greatly increased. Adenomatous goiters are rich in Type IV cells. Hürthle cells, which occur in many abnormal thyroids, were also identified.

RESULTS

In the accompanying Table are listed the major histochemical differences found, based upon present-day interpretations of the

Major Histochemical Distinctions in Frozen-Dried Human Thyroid Tissues

Cell Cytoplasm	Normal	Hyperplasia	Adenomatous
Ribonucleoproteins	Moderate	Increased	Decreased
Mucoproteins	Superficial layer	Superficial layer	None
Protein-bound sulfhydryls	Slight	Slight	Increased,* granules
Pyroninophilic granules	Moderate	Decreased	Increased
Peroxidase	Slight	Moderate	None
Follicular Colloid			
Ribonucleoproteins	Slight, variable	Increased, marginal	Reduced
Mucoproteins	Present	Variable, decreased	Variable, increased
Sulfhydryl compounds	None	Moderate	Weak or moderate
Potassium	None	None	Present
Soluble after mild hydrolysis	None	Marginal in follicles	Central in follicles
Blood Vessels			
Number †	Normal	Marked increase	Decrease

* Also Hürthle cells.
† Demonstrated by alkaline phosphatase reaction.

meanings of the reactions observed. Individual cytologic variations in the histochemical reactions of the major cell types are considered below.

Thyroid Cells

(*a*) *Normal.*—Significant staining was found with four procedures.

1. Methyl green pyronine stained nuclei with methyl green in proportion to their content of deoxyribose nucleoprotein chromatin content. Cytoplasm was diffusely stained with pyronine in all types of thyroid cells, and pyronine-positive granules were present in cells of Types I and IV. Tests with ribonuclease to remove ribonucleoprotein eliminated most of the diffuse cytoplasmic staining. The granules were not altered; hence their pyroninophilia could not be ascribed to nucleoprotein, and their identity remains uncertain.

2. Periodic acid-Schiff stains for glycoproteins and mucoproteins generally colored a superficial rim of cytoplasm in Type I and II cells lining microfollicles.

3. Sulfhydryl groups of proteins were demonstrated in small amounts in the cytoplasm of cells of Types I and IV.

4. Peroxidase test was weakly positive in the cytoplasm of Type I cells, moderate in Type II cells, and negative in Type IV cells. Cell membranes of Type IV cells were artifactually outlined by this procedure.

(*b*) *Hyperplasia.*—Compared with normal, the methyl green pyronine stain showed an increased pyroninophilia of the cytoplasm of Type II cells. When treated with ribonuclease, the abundant nucleoprotein material was removed.

Periodic acid-Schiff and sulfhydryl stains were normal. Type I and IV cells had a somewhat increased cytoplasmic protein-bound sulfhydryl content compared with Type II cells. This likely was partly ascribable to increase of succinic dehydrogenase. Nuclei of all the cell types were shown with the potassium stain (Fig. 1). No intracellular alkaline or acid phosphatases were demonstrated.

Frozen-dried tissues from cases of thyroid hyperplasia, when stained with the above techniques or with hematoxylin and eosin, did not demonstrate any Bensley-type cytoplasmic vacuoles, usually considered typical of Type II cells.[5] Routine preparations, however, showed abundant Bensley vacuoles. Possible effects upon the histochemical reactions of the preoperative medications could not be determined for lack of suitable untreated case material.

(*c*) *Adenomatous Goiter.*—Methyl green pyronine and sulfhydryl stains demonstrated a heavier than normal coloration of the cytoplasm of the predominant Type I and IV cells. The staining was usually granular and was not affected by ribonuclease treatment. The potassium test demonstrated nuclei prominently. Other stains were negative.

In frozen-dried tissues stained with hematoxylin and eosin, apically located poorly stained cytoplasmic droplets or vacuoles were regularly demonstrated in the Type I cells of adenomatous goiters but were not evident after Zenker fixation.

Follicular Colloid

(*a*) *Normal.*—Periodic acid-Schiff stain regularly colored the colloid strongly.[6]

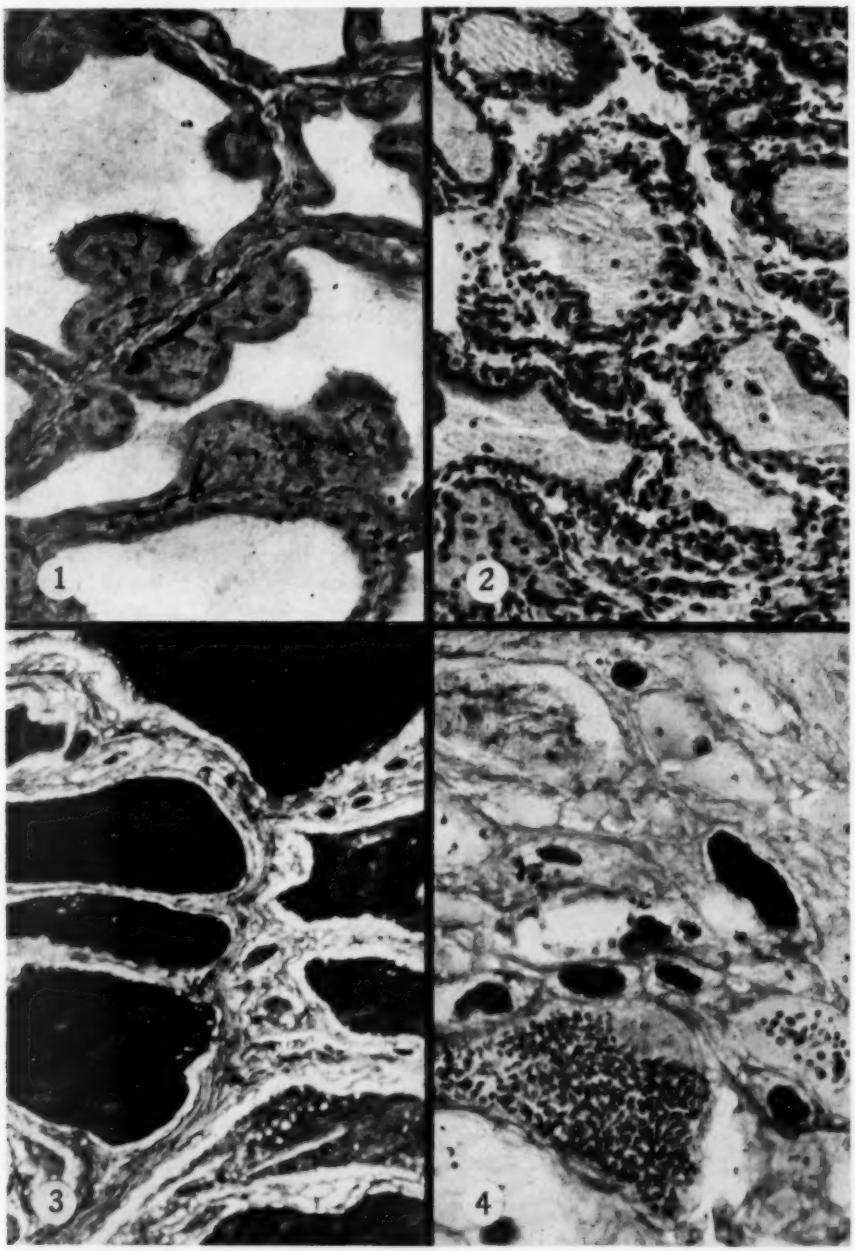


Fig. 1.—Potassium stain from a thyroid with primary hyperplasia and moderate involution, with demonstration of the nuclei by this procedure. #132101. Mag. × 200.

Fig. 2.—Hematoxylin and eosin stained specimen of frozen-dried thyroid, in primary hyperplasia. No scalloping of colloid is found above the Type II columnar cells. #148488. Mag. × 200.

Fig. 3.—Periodic acid-Schiff stain of same specimen as shown in Figure. 2. Vacuolar scalloping of the peripheral colloid is now evident. Mag. × 200.

Fig. 4.—A portion of adenomatous goiter stained with periodic acid-Schiff method, showing granular PAS-positive material in the colloid. #132796. Mag. × 200.

Methyl green pyronine occasionally stained with pyronine the contents of a single or scattered follicles. Artifactual blackening occurred sometimes in preparations for alkaline phosphatase. Other stains were negative. When frozen-dried, the colloid on microscopic slides had at times a striated appearance. No vacuoles or peripheral scalloping were observed.

(*b*) *Hyperplasia.* — Periodic acid-Schiff stains were irregular and locally decreased. The method for both sulfhydryls and pyronine ordinarily stained significant amounts of intrafollicular colloid. The pyroninophilia was found particularly at the colloid margins above Type II cells and was shown to be ribose nucleoprotein by removing it with ribonuclease. The basophilia of thyroid colloid also can be shown by other methods to reflect its nucleoprotein content,[4] although not in formalin-fixed tissue.[6]

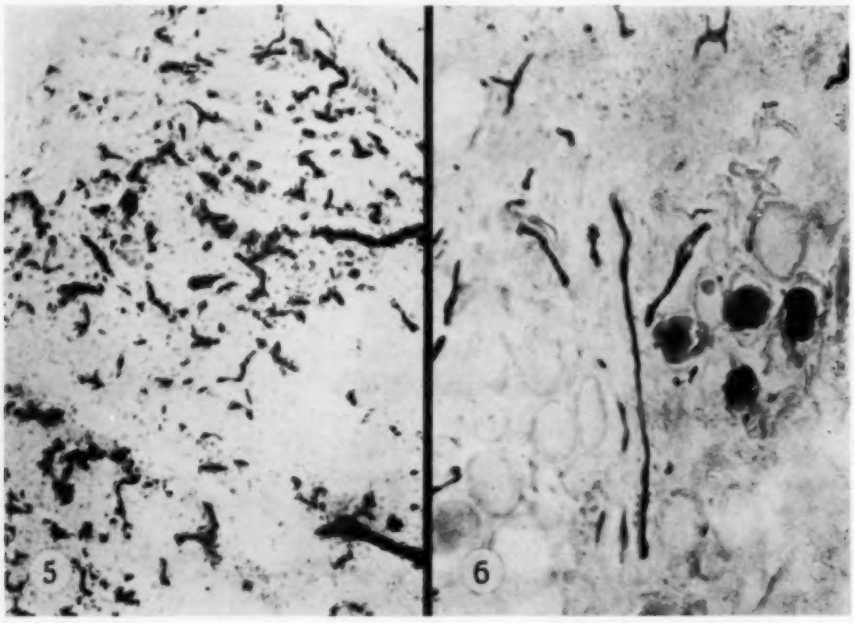
The peripheral scalloping of intrafollicular colloid in thyroid hyperplasia was not found in frozen-dried material stained with hematoxylin and eosin. Usually the scalloping was evident in periodic acid-Schiff preparations, but not with the other histochemical procedures, reflecting a local dissolution after mild hydrolysis with periodic acid (Figs. 2 and 3).

(*c*) *Adenomatous Goiter.*—With periodic acid-Schiff technique the variation of staining between follicles was greater and the general staining stronger than normal in two-thirds of the group. PAS-positive granules were present in the colloid of some microfollicles (Fig. 4). Pyroninophilia of colloid was frequent, particularly in microfollicles. Based on ribonuclease digestion, up to half of this staining was ascribable to ribonucleoprotein. Sulfhydryls were stained in the colloid weakly or moderately, and potassium stains appeared positive.

With frozen-dried material, large central vacuoles were found in intrafollicular colloid of several cases; and at other times the colloid appeared unusually granular. Central vacuoles were prominent in the PAS preparations. Polyhedral crystals, similar in appearance to those previously identified in

Fig. 5.—Alkaline phosphatase method, demonstrating numerous blood vessels in primary thyroid hyperplasia. #148624. Mag. × 125.

Fig. 6.—Normal thyroid gland, blood vessels as shown by alkaline phosphatase technique, for comparison with Figure 5. Mag. × 125.



thyroid colloid as calcium oxalate,[16] were found in 3 of 18 adenomatous glands.

Hürthle Cells

The Hürthle cells had no distinctive histochemical staining and were not so easily identified as in routine preparations. There was a moderate to strong cytoplasmic reaction for protein-bound sulfhydryls in Hürthle cells.

Stroma and Blood Vessels

Both basement membranes and vessel walls stained strongly with PAS in all three groups. No basement membrane abnormalities, such as occur in chronic thyroiditis,[18] were observed. As in other tissues, alkaline phosphatase was abundant in blood vessel walls and provided a striking demonstration of the vascular pattern.

In primary thyroid hyperplasia there was a marked increase in vascularity proportional to the severity (Fig. 5). In instances of involuted thyroid hyperplasia, the regions of colloid storage had a normal (Fig. 6) or subnormal vascular supply. Where increased numbers of Type II cells remained, vessels were still abnormally prominent. Adenomatous goiter characteristically was quite avascular, as shown with the stain for alkaline phosphatase. Relatively more numerous vessels were found in foci of secondary hyperplasia and adjacent to groups of Hürthle cells in specimens of nodular goiter.

COMMENT

By its histochemical reactions alone the recognition of primary thyroid hyperplasia would be possible. Translated into functional terms, the hyperplastic thyroid glands showed evidence of an abnormally increased metabolic activity involving blood vessel walls, thyroid parenchyma, and colloid. Peroxidase enzyme activity, which is involved in transforming iodide to iodine,[5] was greater than normal. Increased amounts of nucleoprotein were found in follicles. Stronger staining for protein-bound sulfhydryls in the colloid might reflect an increased concentration of oxidation-reduction enzymes associated with hormone reabsorption.[2] Decreased PAS stain would suggest an enhanced mucinase activity in the colloid prior to its resorption. Marginal colloid was unusually soluble after periodic acid hydrolysis, and the resultant peripheral colloid scalloping would indicate increased proteolytic activity preceding colloid resorption. It is notable that although the scalloping of colloid in thyroid hyperplasia is an artifact of chemical fixation, it reflects an actual increase in intrafollicular proteolysis that is characteristic of hyperthyroidism.[5]

Adenomatous goiters by histochemical tests showed indications of a generally sluggish metabolic activity, with foci of normal or increased metabolism. Peroxidase activity of parenchyma was absent. The Type IV thyroid cells, although containing active sulfhydryl groups and, according to Goddard and Seligman,[8] considerable succinic dehydrogenase activity, were not efficiently elaborating colloid. Some intrafollicular colloid in large follicles of adenomatous goiters was stored for an abnormally long time, as suggested by the central vacuoles, the granular pyroninophilic material, strong PAS staining, and the increased potassium content.

Indirectly these histochemical tests supported the importance of proteolytic enzymes and mucinases in the hydrolysis of intrafollicular colloid preparatory to its accelerated resorption and internal secretion in hyperthyroidism. Associated as these increased enzymatic activities were with peripherally scalloped intrafollicular colloid, a therapeutically induced involution with increased colloid storage seemed to be excluded as responsible. The study of enzymes also suggested an abnormally slow turnover of the colloid reservoir in adenomatous goiters, an observation previously made by autoradiography.[19]

Additional enzymes that others have located histochemically in thyroid parenchyma include phosphatases,[4] 5-nucleotidase,[18] and β-D-glucuronidase.[17] Concentrations of intracellular phosphatases were insufficient for positive staining in our human thyroid material, although reported to stain in rats.[4]

SUMMARY

Using seven histochemical stains the reactions of human thyroid tissues from cases of primary hyperplasia and adenomatous goiter were compared. Normal glands were available as controls. Primary thyroid hyperplasia had histochemical indications of stimulated metabolic activities, including increased intracellular peroxidase enzymes and nucleoprotein synthesis. Enhanced activity in intrafollicular colloid of proteolytic and mucolytic enzymes contributed to accelerated colloid resorption. Adenomatous goiters had an evidently depressed metabolism, with evidence of sluggish turnover of the intrafollicular colloid. Cytologic morphology provides a sound basis for interpreting thyroid activity.

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Analysis of Focalized Pulmonary Granulomas Due to Coccidioides Immitis

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The "coccidioidal pulmonary residual" is an all-inclusive term applied to the different types of focalized lesions remaining in the lung parenchyma, either temporarily or permanently, following the resolution of the acute pneumonitis, either clinical or "inapparent," due to the fungus Coccidioides immitis. These focalized lesions can be divided into three major groups on the basis of their gross structural characteristics, the criteria of which can be determined clinically by roentgenologic examination. These are: (1) the solid fibrocaseous granuloma, (2) the fibrocaseous granuloma with cavitation, and (3) the linear fibrotic scar. It is the purpose of this paper to discuss the first two types of focalized lesions only, since the linear fibrotic scar represents the final stage in healing and probably is of no further clinical significance. Before doing so, a brief historical review is presented concerning the coccidioidal pulmonary residual and its relationship to the other phases of coccidioidomycosis.

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The opinions or assertions contained herein are the private ones of the writers, and are not to be construed as official or reflecting the view of the Navy Department or the Naval Service at large.

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HISTORICAL REVIEW

The first authentic case of a coccidioidal pulmonary residual was reported by Dickson in 1915.[1] The lesion was found in the base of the right lung of a 59-year-old white man who died of carcinoma of the stomach. The microscopic examination recorded by Ophüls was as follows:

> A section of the lung shows large old caseous areas surrounded by dense fibrous tissue. At the edge of the caseous material there are a number of double-contoured capsules of the Oidium coccidioides. The majority of them are empty, but some contain a little granular protoplasm. Diagnosis: Healed coccidioidal granuloma of the lung.

No tubercle bacilli could be demonstrated in the lesion, and no other foci of coccidioidal granuloma were found.

During the next 22 years the great concern of clinician and pathologist alike was the disseminated form of the disease, which was then referred to as "coccidioidal granuloma." This was considered to be a disease with a mortality of approximately 50%.[2] That a primary coccidioidal pneumonitis without dissemination existed was not generally appreciated despite Dickson's paper in 1915. As early as 1905, however, Ophüls [3] had suspected that there was a primary pulmonary infection in "coccidioidal granuloma," probably due to inhalation of the fungus. Expressing this opinion again in 1929, Ophüls [4] stated that the infecting agent was evidently the spore which developed on

the aerial hyphae of the mycelium of the fungus and that, in cases that had come to autopsy, it was possible to find an old primary focus in the lungs. Two months later Ophüls [5] made the following prophetic statement: "Spontaneous recovery or at least quiescence of the disease undoubtedly does occur."

During this same period an acute influenzal-like ailment of self-limited type, which was associated with erythema nodosum, was well-known in the lower San Joaquin Valley as "valley fever." The etiology of this clinically benign ailment, however, had never been established, until Dickson [6] in 1937 reported that Coccidioides immitis was not only the etiological organism of "coccidioidal granuloma" but also that of "San Joaquin Valley Fever." Dickson and Gifford [2] in 1938 stated that the acute coccidioidal pneumonitis sometimes was not associated with erythema nodosum and, hence, did not come under the original classification of "San Joaquin Valley Fever." They further stated that dissemination occurred in a few patients who had had erythema nodosum. As a result of their studies they suggested that the name "coccidioidomycosis" be used to include all types of infection due to the fungus C. immitis.

In 1943 Smith [7] laid down the following classification of the manifestations of coccidioidomycosis:

(1) Initial or primary infection
  (*a*) Inapparent or asymptomatic form
  (*b*) Acute respiratory, "influenzal," or "pneumonic" form
  (*c*) Either of above types associated with erythema nodosum or erythema multiforme
  (*d*) Pulmonary cavity form
(2) Progressive, "secondary" disseminated infection

Consequently considerable interest was stimulated in the study of the pulmonary residuals in cases of arrested primary coccidioidomycosis. In 1939 Cox and Smith [8] reported four cases in which the arrested coccidioidal granulomas were incidental findings at autopsy. Three lesions were located in the lung parenchyma, and one in a large hilar lymph node. In all cases spherules, including a few with endospores, were found in the histologic sections of the fibrocaseous lesions. C. immitis was also cultured from the calcified pulmonary lesion in Case 4, in which there was evidence of minimal dissemination of the coccidioidal infection.

In 1945 Butt and Hoffman [9] reported the results of their deliberate search at autopsy for coccidioidal pulmonary residuals. Thirty-six Santa Fe Railroad employees, who had made repeated trips through California's lower San Joaquin Valley, had been skin-tested with both coccidioidin and tuberculin prior to death. Eleven were positive coccidioidin reactors and had multiple calcified lesions both in the lungs and in peribronchial lymph nodes. Although spherules were found in the healed lesions of eight patients, in only five were endosporulating forms demonstrated. Since budding forms characteristic of the smaller spherules of Blastomyces dermatitidis were not demonstrated in the lesions of the other three patients, the authors were inclined to accept the presence of spherules without endospores as presumptive but not unequivocal evidence of healed coccidioidomycosis. They emphasized that identification of endosporulating spherules is necessary in order to distinguish C. immitis from B. dermatitidis. The lesions of the remaining three patients with a positive coccidioidin skin test contained no demonstrable spherules.

Since this time much of voluminous literature on the various types of coccidioidal pulmonary residuals concerns cases with non-resected cavities in which the etiology was established by positive sputum cultures,* or cases in which the lesions were surgically resected,† or cases based on x-ray studies of the postpneumonic phase of the disease in which the diagnosis was originally established at the time of the acute pneumonitis by positive sputum cultures.‡

* References 10 through 15.

† References 15 through 23.

‡ References 24 and 25.

SUMMARY OF CLINICAL HISTORIES

Eleven cases are presented. All patients were men, 10 of Caucasian descent and one of Mexican parentage. Their ages ranged from 21 to 47 years. Six patients probably had acquired their primary coccidioidal infection in Southern California, one in the San Joaquin Valley, and four in Arizona (Tables 1 and 2). The four solid lesions were discovered by routine chest x-ray in asymptomatic patients, none of whom ever had a history of an acute pneumonitis suggestive of the primary infection of coccidioidomycosis. Despite thorough clinical investigation, the exact nature of these "solitary pulmonary nodules" could not be established with certainty, and hence they were surgically resected, three by lobectomy (Cases 1, 2, and 3, Table 1) and one by wedge resection (Case 4, Table 1).§ Five of the seven patients with cavities had a history of an acute pneumonitis. Although the possibility of an acute coccidioidal pneumonitis was clinically suspected at the time, a definite diagnosis by means of positive sputum cultures had not been established. Subsequently these patients had sporadic episodes of cough, hemoptysis, low-grade fever, and related symptoms, as well as radiologic evidence of a persisting cavity for periods of 4 to 30 months (Cases 1, 2, 3, 4, and 7, Table 2). Although the sixth patient never had a history of an acute pneumonitis, subsequent symptoms of cough and hemoptysis led to medical investigation, with discovery of the cavity by chest x-ray (Case 5, Table 2). The seventh patient never had a history of an acute pneumonitis and was asymptomatic at the time of discovery by routine chest x-ray (Case 6, Table 2). Wedge resection of the cavity was performed in all seven cases. Following postoperative recovery all 11 patients were discharged from the hospital either to full duty or to civilian status.

§ Rolle, C., Jr.; Alznauer, R. L., and Pierce, W. F.: Problem of the "Solitary Pulmonary Nodule," unpublished data.

TABLE 1.—*Summary of the Solid Pulmonary Lesions of Focalized Coccidioidomycosis*

Case	Date of Discovery and Probable Geographical Site of "Inapparent" Coccidioidal Pneumonitis	Size and Location of Solid Lesion	Satellite Tubercles		Location of Spherules		Skin Tests			Coccidioidal Complement-Fixation Reaction
			Few	Many	Satellite Tubercles	Caseous Material	Coccidioidin 1:100	PPD	Histoplasmin 1:100	
1. 37 yr.; admitted 2-15-52; surgery 4-9-52	Discovered on 11-26-50; residence in Southern California 19 yr.	2.5 cm. LLL	..	+	+	+	+	± 1 +	+	1:2 ++ (3-12-52)
2. 36 yr.; admitted 1-7-52; surgery 4-16-52	Discovered on 4-9-51; Phoenix, Ariz.	2.0 cm. LUL	+	..	—	+	+	± 1 +	+	1:2 1:4 1:8 1:16 +++ ++ ++ + (2-1-52)
3. 21 yr.; admitted 4-21-52; surgery 6-11-52	Discovered 1 yr. prior to hospitalization; Phoenix, Ariz.	2.5 cm. RUL	..	+	+	—	1:100 — 1:10 +	± 1 — ± 2 —	+	Negative (5-11-51)
4. 22 yr.; admitted 11-20-52; surgery 12-9-52	Date of discovery unknown; residence in Los Angeles with several trips in San Joaquin Valley	1.8 cm. RLL	..	+	+	+	+	± 1 — ± 2 +	+	1:2 1:4 1:8 ++++ ++++ ++ (12-2-52)

All four patients with solid fibrocaseous granulomata due to Coccidioides immitis were asymptomatic, and the lesions were discovered by routine chest x-ray as "solitary pulmonary nodules." None of the patients had a past history of an acute pneumonitis suggestive of the primary infection of coccidioidomycosis.

TABLE 2.—*Summary of the Cavitary Pulmonary Lesions of Focalized Coccidioidomycosis*

Case	Time and Probable Geographical Site of Acute Pneumonitis	Size and Location of Cavity	Patent Bronchus	Satellite Tubercles		Location of Spherules			Mycelial Form in Cavity	Skin Tests			Coccidioidal Complement-Fixation Reaction
				Few	Many	Satellite Tubercles	Caseous Material	Bronchial Lumen		Coccidioidin 1:100	PPD	Histoplasmin 1:100	
1. 47 yr.; admitted 1-22-52; surgery 4-23-52	Sept., 1949; San Diego, Calif.	1.5 cm. LUL	—	+	—	—	+	—	—	1:1000 +	—	—	1:2 ++++ (11-7-49) 1:2 ++++ 1:4 + (2-6-52)
2. 28 yr.; admitted 6-3-52; surgery 6-5-52	July, 1952; San Diego, Calif.	2.0 cm. RLL	—	+	—	—	+	—	—	+	—	+	1:2 ++ (4-11-52) 1:2 +++ 1:4 + (8-1-52)
3. 25 yr.; admitted 4-21-52; surgery 12-4-52	May, 1951; Tucson, Ariz.	1.8 cm. RLL	+	—	+	+	+	+	—	1:1000 +	‡2 —	—	Negative (10-31-52)
4. 36 yr.; admitted 11-6-52; surgery 2-9-53	June, 1952; Phoenix, Ariz.	3.5 cm. RLL	+	+	—	—	+	—	+	+	‡1 — ‡2 +	—	Negative (12-25-52)
5.* 26 yr.; admitted 2-2-53; surgery 3-12-53	Discovered in May, 1952, due to symptoms. Never had acute pneumonitis. "Inapparent" infection probably in San Joaquin Valley (home in Madera, Calif.)	2.8 cm. RLL	+	+	—	+	+	—	+	1:100 — 1:10 +	—	—	1:2 ++++ 1:4 ++++ 1:8 ++++ (2-25-53) 1:2 ++++ 1:4 ++++ 1:8 + (5-29-53)
6.* 21 yr.; admitted 4-20-53; surgery 5-18-52	Asymptomatic. Never had acute pneumonitis. Probably San Diego or Long Beach, Calif. Discovered March, 1953.	3.0 cm. LLL	+	+	—	—	+	+	+	+	‡2 —	+	1:2 ++++ (4-25-53)
7. 22 yr.; admitted 3-10-53; surgery 5-11-53	April, 1950; San Diego, Calif.	2.5 cm. RUL	+	—	+	+	+	—	—	1:100 — 1:10 +	‡1 +	+	Negative (4-24-53)

Six of the seven patients with cavitary fibrocaseous granulomata due to Coccidioides immitis had symptoms leading to medical investigation with discovery of the cavities by chest x-ray. Only five patients, however, had a past history of an acute pneumonitis suggestive of but not proved to be the primary infection of coccidioidomycosis. The seventh patient not only was asymptomatic at the time the cavity was discovered by routine chest x-ray but also never had a past history of an acute pneumonitis.

* Cultures on Sabouraud's medium were positive for C. immitis in these two cases.

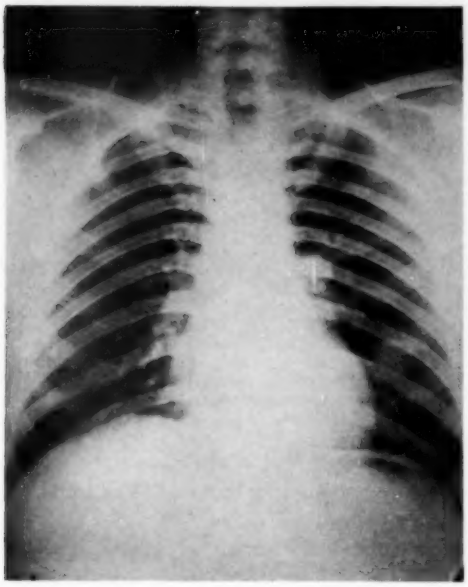
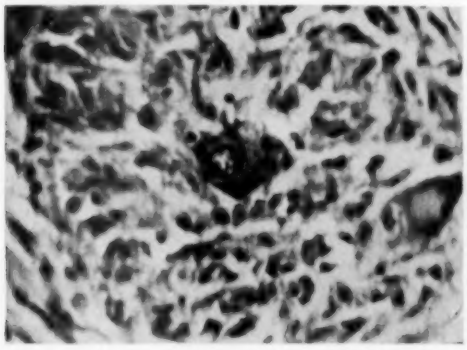


Fig. 1 (Case 1, Table 1).—Chest x-ray, postero-anterior view, showing the "solitary pulmonary nodule" in the lower lobe of the left lung.

COCCIDIOIDIN SKIN TEST

All 11 patients had a positive coccidioidin skin test, 2 in 1 : 1000 and 6 in 1 : 100 dilution. In the other three patients the test was negative in 1 : 100 dilution, but was positive when repeated in 1 : 10 dilution. In 1948 Smith, Beard, and Saito [26] stated that 10% of coccidioidin reactors have a negative skin test in 1 : 100 dilution but a positive test in 1 : 10 dilution.

Fig. 2 (Case 1, Table 1).—Photomicrograph showing a double-contoured spherule within a multinucleated giant cell of a satellite epithelioid tubercle; × 440.



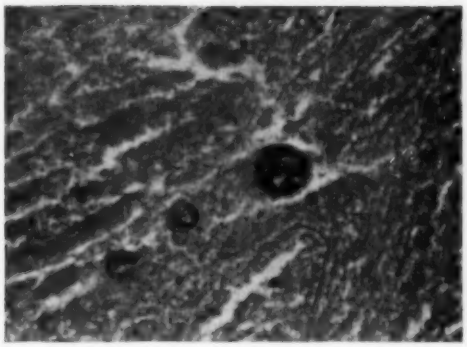


Fig. 3 (Case 1, Table 1).—Photomicrograph showing three spherules of varying size within loose caseous material; × 440.

In only three cases, however, was the coccidioidin skin test positive, while both the histoplasmin and tuberculin skin tests were negative (the material used was purified protein derivative, hereafter referred to as PPD in Tables 1 and 2). The other eight patients also had a positive reaction to either or both histoplasmin and tuberculin (Tables 1 and 2). Hence the results of the skin tests were unreliable for a clinical interpretation of the pulmonary lesions as probable coccidioidal residuals.

There are apparently no cross reactions between coccidioidin and tuberculin according to those investigators who have made extensive experimental [27] and clinical studies with these antigens.‖ Furthermore, Smith [7]

‖ References 7, 9, and 28.

Fig. 4 (Case 1, Table 1).—Photomicrograph showing a large spherule containing endospores within loose caseous material. Endosporulation is the critical feature for the interpretation of the organism as that of Coccidioides immitis. × 440.

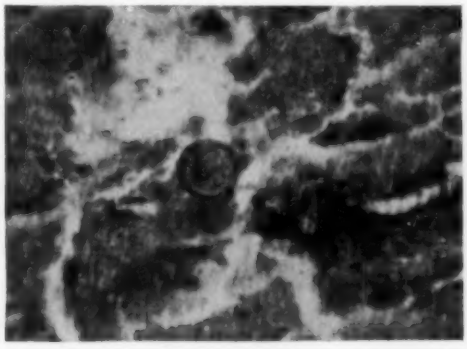


Fig. 5 (Case 2, Table 1).—Photomicrograph showing a large degenerated spherule in which endospores are still recognizable. Note the wrinkling of the capsule. ×440.

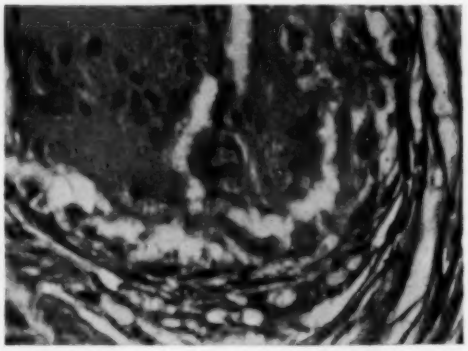


Fig. 7 (Case 4, Table 1).—Photomicrograph showing a spherule in the multinucleated giant cell of a satellite epithelioid tubercle. × 440.

states that the patient usually retains his specific allergy and that people who have left an endemic area nearly 30 years before may still react vigorously to coccidioidin.

SEROLOGIC TEST FOR COCCIDIOIDOMYCOSIS

Coccidioidal complement-fixation tests on sera from all patients were performed in the facilities provided at the University of California, School of Public Health, by the Commission on Acute Respiratory Diseases of the Armed Forces Epidemiological Board. The serologic titers were considered by Smith ¶ to be diagnostic of coccidioidal infection in six cases, equivocal in one case, and negative in the remaining four cases (Tables 1 and 2). Smith, Beard, and Saito [26] regard a full 4+ reaction in 1:2 dilution as diagnostic, either a 2+ or 3+ reaction in 1:2 dilution as "equivocal," and a 1+ reaction as negative.

As a supplement to the positive coccidioidin skin test, the results of the complement-fixation tests lent considerable support to the clinical impression that the pulmonary lesions were probably coccidioidal residuals in three additional cases. One of these had a positive skin reaction to histoplasmin, and two had positive reactions to both histoplasmin and tuberculin (Tables 1 and 2).

The five patients with either a negative or equivocal serologic test also had positive skin reactions to either or both histoplasmin and tuberculin. In this situation Smith [29] states

¶ Smith, C. E.: Personal communications.

Fig. 6 (Case 3, Table 1).—Photomicrograph of a satellite epithelioid tubercle adjacent to the hyalinized fibrous capsule surrounding the caseous center of a solid focalized granuloma. Note the spherule in the lower portion of the large multinucleated giant cell. The adjacent fibrous capsule contains numerous lymphocytes, and caseous material is present in the lower right hand corner of the field. × 100.

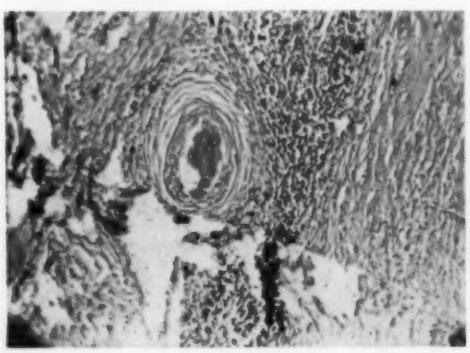
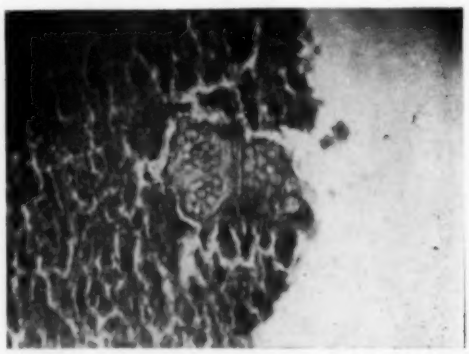


Fig. 8 (Case 4, Table 1).—Photomicrograph of the caseous center of the main lesion, showing two large degenerated endosporulating spherules with wrinkled and distorted capsules; × 440.



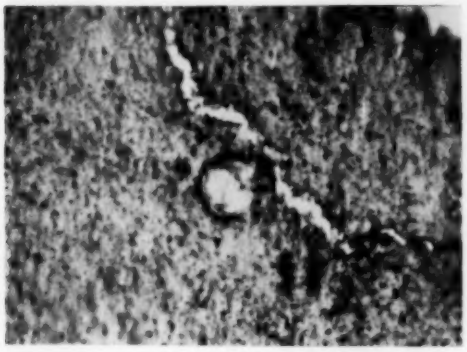
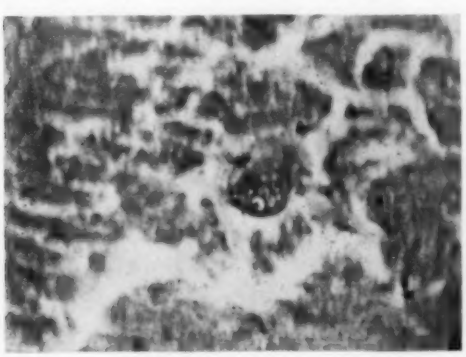


Fig. 9 (Case 1, Table 2).—Photomicrograph showing a degenerated spherule which is merely an empty shell surrounded by caseous material; × 440.

that the diagnosis can only be made by the demonstration of the etiologic organism.

Although the number of cases in this series is too small to be statistically significant, the results generally are in keeping with the conclusions arrived at by Smith, Beard, and Saito.[26] There was fixation of complement in approximately three-fifths of their cases with coccidioidal cavities. This was usually in a low titer. A total of 94% fixed complement to titers of 1 : 8 dilution or less, and 50% had complete fixation only in 1 : 2 dilution. In the remaining two-fifths the results were either negative or "equivocal." In addition they considered the relatively low titers in cases with focalized cavities to be in striking contrast to the high titers observed in cases with the disseminated form of coccidioidomycosis. Seven-eighths of these cases fixed complement to a serum dilution higher than 1 : 16.

Fig. 10 (Case 2, Table 2).—Photomicrograph showing a degenerated spherule in which the endospores are still recognizable; × 440.



PATHOLOGIC FINDINGS

The four solid lesions ranged from 1.8 to 2.5 cm., and the seven cavitary lesions from 1.5 to 3.5 cm. in maximal size. One solid and one cavitary lesion were located in the left lower, left upper, and right upper lobes, while one solid and four cavitary lesions were located in the right lower lobe (Tables 1 and 2). Both types frequently were lobulated in contour. The peripheral capsule ranged

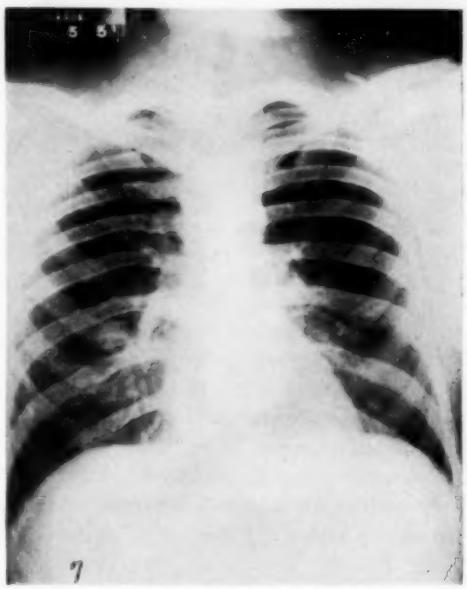


Fig. 11 (Case 3, Table 2).—Chest x-ray, postero-anterior view, showing the "solitary pulmonary nodule" in the lower right lung field (August, 1951).

from 0.2 to 0.4 cm. in thickness and was firm and gray-white. Microscopically this was composed of hyalinized fibrous tissue containing some lymphocytes and occasional multinucleated giant cells. The inner lining of four cavitary lesions was partially composed of vascular granulation tissue, which showed varying degrees of nonspecific acute inflammatory reaction and even foci of fresh hemorrhage (Cases 3, 4, 5, and 7, Table 2). Yellow to gray caseous material, much of which was soft and semidiffluent, filled the centers of the solid lesions. Microscopically

Fig. 12 (Case 3, Table 2).—Chest x-ray, postero-anterior view, showing subsequent conversion of the solid lesion into a cavity one year later (August, 1952), four months prior to surgical resection.

Fig. 14 (Case 4, Table 2).—Chest x-ray, postero-anterior view, showing a thin-walled cavity in the right lower lung field.

this was predominantly loose and amorphous, but in some areas ghost-like remnants of pulmonary parenchyma were still recognizable. Some cavitary lesions had largely empty centers with only shreds of caseous material clinging to the inner lining. In four cavities microscopic examination showed that the loose caseous material was intermingled with purulent exudate (Cases 3, 4, 5, and 7, Table 2).

A communicating bronchus was grossly identifiable in one case (Case 6, Table 2). A small cavity was eccentrically located adjacent to this communicating bronchus. Firm laminated caseous material, in which minute calcium deposits were seen microscopically, occupied the remainder of the center. In the histologic sections, however, a patent communicating bronchus was demonstrable in four additional cavitary lesions (Cases 3, 4,

Fig. 13 (Case 3, Table 2).—Photomicrograph showing numerous double-contoured spherules which are closely clumped together in the caseous material; × 440.

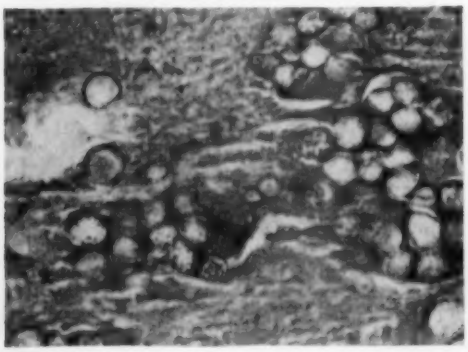
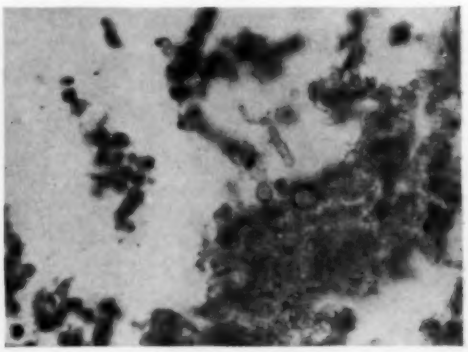


Fig. 15 (Case 4, Table 2).—Photomicrograph showing the mycelial form of Coccidioides immitis in the cavity. Note the septate hyphae with the arthrospores. × 440.



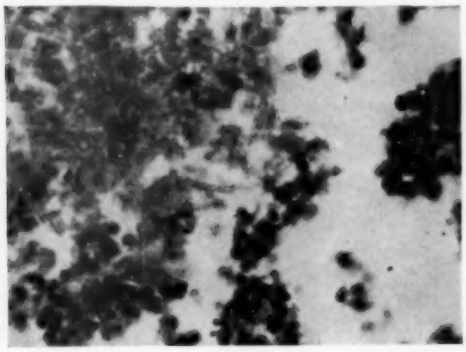
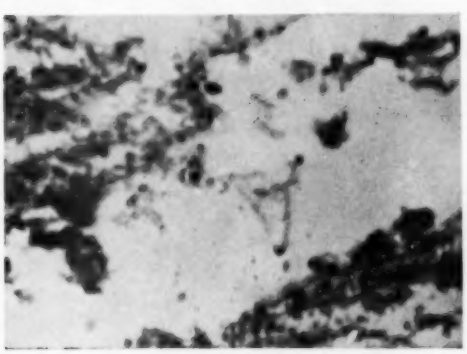


Fig. 16 (Case 5, Table 2).—Photomicrograph showing the mycelial form of C. immitis in the upper and purulent exudate in the lower portion of the field. Two small spherules are also present. × 440.

5, and 7, Table 2). In two cases there was focal squamous metaplasia of the mucosa lining the communicating bronchus (Cases 3 and 6, Table 2).

The pulmonary parenchyma adjacent to the main lesions frequently showed considerable fibrous thickening of the alveolar septae. Although only occasionally recognizable in the gross specimen, microscopically at least a few adjacent satellite lesions were found in all cases. These were numerous about three solid (Cases 1, 3, and 4, Table 1) and two cavitary lesions (Cases 3 and 7, Table 2). The satellite lesions were typical epithelioid tubercles, which frequently contained multinucleated giant cells. Some were discrete and others confluent, and small caseous foci were sometimes present in the latter.

Fig. 17 (Case 5, Table 2).—Photomicrograph showing the septate hyphae of the mycelial form of C. immitis in the middle of the field. × 440.



A few had the appearance of small hyalinized fibrous nodules.

The characteristic double-contoured spherules of C. immitis were demonstrable in all cases in the routine hematoxylin and eosin sections, and we did not find it necessary to resort to the periodic acid-Schiff stain. The critical feature was the discovery of at least a few classic endosporulating spherules in each lesion. In the occasional lesion in which the organisms were sparse and consisted mostly of empty shells, the discovery of a rare endosporulating spherule did require careful search of numerous sections. In one instance (Case 2, Table 2), diagnostic spherules were found in the sections of only 1 of 16 blocks representing the totally embedded lesion. It was our experience, however, that the majority of lesions contained many typical organisms, and hence the correct etiologic diagnosis was established with relative ease.

Fig. 18 (Case 6, Table 2).—Chest x-ray, posteroanterior view, showing a discrete nodule with eccentric cavitation in the left midlung field.

In the four solid granulomata the organisms were found both in the central caseous material and in the satellite lesions in two

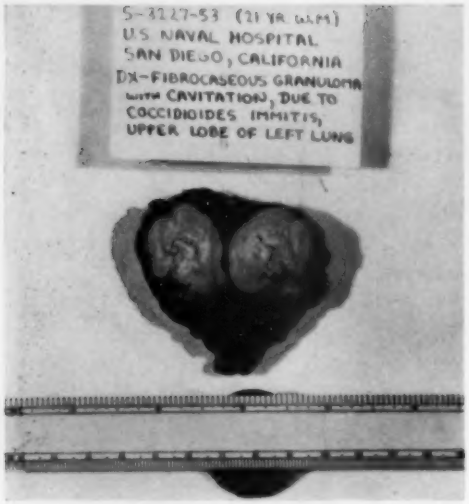
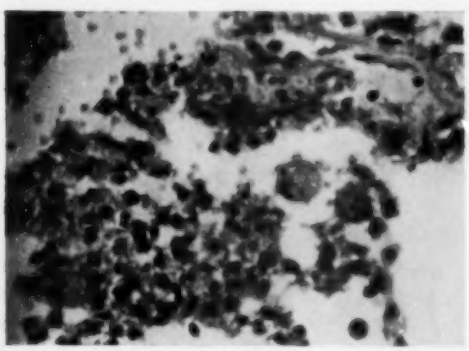


Fig. 19 (Case 6, Table 2).—Photomicrograph of the gross specimen showing the lobulated fibrocaseous granuloma with eccentric cavitation in the lower portion at the site of the communicating bronchus. Note the compact laminated character of the caseous material occupying the remainder of the center of the lesion.

cases (Cases 1 and 4, Table 1), only in the satellite lesions in one case (Case 3, Table 1), and only in the central caseous material in the fourth case. The satellite lesions were sparse about the latter lesion. The organisms were found in the caseous material of the cavity in all seven cases, as well as in the lumen of the communicating bronchus in two (Cases 5 and 6, Table 2). They were also found in the satellite lesions in three cases (Cases 3, 5, and 7, Table 2), in only two of

Fig. 20 (Case 6, Table 2).—Photomicrograph showing a double-contoured spherule surrounded by numerous polymorphonuclear leucocytes; × 440.



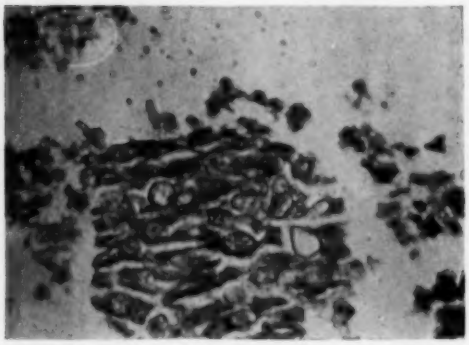


Fig. 21 (Case 6, Table 2).—Photomicrograph showing the mycelial form of C. immitis in the small eccentrically located cavity. Note the septate hyphae with arthrospores. × 440.

which were such lesions numerous. In the satellite lesions the spherules were most frequently seen within the multinucleated giant cells. Occasionally they were also found in the small caseous foci, if present.

Regional peribronchial lymph nodes were available for histologic study in only four cases (Cases 2 and 3, Table 1, and Cases 4 and 6, Table 2). In only two did they contain epithelioid granulomata, and no spherules could be found (Case 2, Table 1, and Case 6, Table 2).

Of great interest was the discovery of the mycelial form of C. immitis in three of the

Fig. 22 (Case 7, Table 2).—Photomicrograph showing a double-contoured spherule within a multinucleated giant cell of a satellite epithelioid tubercle; × 440.

seven cavitary lesions (Cases 4, 5, and 6, Table 2). Septate hyphae with arthrospores were found both in fresh mounts from the cavities immediately after surgical resection and in the actual tissue sections. A patent communicating bronchus was demonstrable in all three lesions. The only cultures in this series were done in two of these cases. Both were positive, with pure growth of C. immitis on Sabouraud's medium.

Duplicate sections of all blocks of all lesions were stained for acid-fast bacilli with Ziehl-Neelsen technique, but none were found. An interesting observation, however, was the fact that the spherules of C. immitis were morphologically as distinct in the Ziehl-Neelsen sections as they were in the hematoxylin and eosin preparations.

DIFFERENTIAL DIAGNOSIS

The focalized fibrocaseous granulomata due to Mycobacterium tuberculosis and due to Histoplasma capsulatum, with or without cavitation, are the two commonest lesions in the lung which must be distinguished from the coccidioidal residual. The solid lesion due to the first organism is well-known as the so-called "tuberculoma." The focalized pulmonary lesions of histoplasmosis, however, have only recently been recognized and described as such in the literature.# One important point in regard to these lesions warrants mention. Puckett [32] has emphasized that it is virtually impossible to demonstrate H. capsulatum in the hematoxylin and eosin sections of such lesions, and only by utilizing the periodic acid-Schiff stain was he able to demonstrate the tiny capsules of this organism with certainty.

Rare cases of focalized pulmonary granulomata of the fibrocaseous type due to other organisms have been recorded. Good, Clagett, and Weed* have seen two such lesions in which Brucella suis was identified as the etiologic organism by culture. Recently Haugen and Baker † have described such a lesion in which Cryptococcus neoformans was identified as the responsible organism. Parsons ‡ has seen such a lesion in which Geotrichum was identified by culture. This organism was also demonstrated in the tissue sections by means of the periodic acid-Schiff stain.

Our survey of the literature did not reveal any cases in which Blastomyces dermatitidis was definitely demonstrated in the caseous center of such an encapsulated pulmonary granuloma. In one of the cases reported by Schwarz and Baum [36] in 1951, this organism was present in an area of granulomatous pneumonitis surrounding a calcified nodule but was not demonstrated in the caseous center of this encapsulated lesion.

The diagnosis "tuberculoma" too often has been made merely on the basis of the so-called "specific" histopathologic reaction, despite the fact that the tubercle bacillus has not been demonstrated in the fibrocaseous lesion. The tenuousness of such a diagnosis is well illustrated by the results of a recent investigation by Zimmerman § at the Armed Forces Institute of Pathology. A careful study of multiple sections prepared with hematoxylin and eosin, Ziehl-Neelsen, and Gridley stains resulted in the positive identification of the etiologic organism in 28 of 35 so-called "tuberculomas." Six were finally proved to be true "tuberculomas." Three, however, were due to C. immitis and the remaining 19 were due to H. capsulatum.

The correct etiologic diagnosis by the pathologist is not merely an academic matter, but is actually of considerable importance and practical value to both the clinician and to his patient. Good, Clagett, and Weed [34] have emphasized that the postoperative care will be more rational if the etiology of the lesion is known. If the resected pulmonary lesion is due to either C. immitis or H. capsulatum, then nothing further need be done in the way of specific antibiotic therapy. If due to M.

References 30 through 33.

* Reference 34, and Weed, L. A.: Personal communication.

† Reference 35, and Baker, R. D.: Personal communication.

‡ Parsons, R. J.: Personal communication.

§ References 37 and 38.

tuberculosis, however, the clinician will undoubtedly supplement the original surgical procedure with appropriate antibiotic therapy.

BIOLOGIC SIGNIFICANCE OF THE COCCIDIOIDAL PULMONARY RESIDUAL

The coccidioidal pulmonary residual is not necessarily stabilized in its existing form. If patent communication with an adjacent bronchus is established, a solid granuloma may become a cavitary lesion following the evacuation of its caseous contents. This occurred in one of our cases (Case 3, Table 2). Conversely, a cavitary lesion may be converted to a solid granuloma because of obstruction of its communicating bronchus followed by inspissation of its contents due to the reabsorption of fluid. Despite such occasional mechanical fluctuation in its structure, the coccidioidal residual biologically is a relatively bland and innocuous lesion.

In a follow-up report on his cases, Winn [39] states that the coccidioidal cavity closed spontaneously in 25% of 92 patients, that such cavitation had persisted for years without harm to the patient in 10%, and that the cavities had filled and formed solid solitary foci in 6%. In 14 cases surgical resection of the pulmonary lesion was performed.

Winn [39] further states that in his series there has been no dissemination of disease from a residual pulmonary cavity. Smith, Beard, and Saito [26] also state that the risk of dissemination from a coccidioidal cavity is negligible. Winn [11] and Smith || both are of the opinion not only that the arrested residual does not predispose toward dissemination of endogenous origin but also that a lasting immunity to exogenous reinfection must be produced in the host by the primary infection of C. immitis. Otherwise, most of the inhabitants of the San Joaquin Valley sooner or later would develop disseminated coccidioidomycosis with its attendant high mortality rate, if reexposure could cause a second infection. Smith [40] further states that laboratory animals after one infection are resistant to reinfection.

|| References 7 and 40.

Cotton and Birsner [22] are of the opinion that surgical treatment in pulmonary coccidioidomycosis does not cause dissemination of the disease. In their series of 50 cases, Forsee and Perkins [23] also state that they have not observed extrapulmonary dissemination as a complication of pulmonary surgery for coccidioidal residuals.

One complication of residual cavities located in a subpleural position, which warrants mention, is that of spontaneous rupture into the pleural cavity with consequent hydropneumothorax or pyopneumothorax as well as bronchopleural fistula. This occurred in 2.6% of the cases in the series reported by Smith, Beard, and Saito.[26] Seven of 13 patients with this complication had a demonstrable antecedent cavity. Winn [39] also reported spontaneous rupture in 2 of his 92 patients with cavities. Forsee and Perkins [23] had observed this complication in five cases with subpleural cavities, which were not included in their present report. Although the organism can be cultured from the pleural effusion, this complication is of local significance only and apparently carries no inherent danger of widespread dissemination of the disease. Because of fibrous pleural thickening, decortication may ultimately be necessary in some cases.

As already mentioned, the infectious mycelial form of C. immitis was demonstrated in three of the seven cavitary lesions presented. The first such case was described by Forbus and Bestebreurtje in 1946.[41] Unidentifiable hyphae and spores were demonstrated in the exudate of a large apical cavity, in which no spherules were found in the tissue sections. C. immitis was cultured from the pus in the cavity. The authors stated that this was the only such case on record at that time. Subsequently the mycelial form of the organism was demonstrated both in fresh mounts and in tissue sections from six of nine coccidioidal cavities reported by Greer, Forsee, and Mahon in 1949,[20] and in fresh smears from a coccidioidal cavity reported by Weisel and Owen in 1949.[21] In the most recent series from Fitzsimons Army Hospital, Forsee and Perkins [23] stated that

the mycelial form was present in 15 of 30 cavities and in 2 of 20 solid lesions. This phenomenon raises the theoretical possibility that direct person-to-person infection might occur. To the best of our knowledge no such situation has yet been reported in the literature. Such an occurrence would have to be established in a nonendemic area for C. immitis.

COEXISTING COCCIDIOIDOMYCOSIS AND TUBERCULOSIS

Cases of coexisting coccidioidomycosis and tuberculosis in various different combinations have been reported in the literature.¶ Cases of coccidioidal cavities which were apparently secondarily infected by tubercle bacilli are at present apparently rare. Cotton and his associates # have observed three such cases (Cases 1, 2, and 5, in Table 1 of their series). In Case 1 both organisms were demonstrated in fresh smears from the lesion and in tissue sections, in Case 2 they were demonstrated in tissue sections only, and in Case 5 they were demonstrated by cultures and in tissue sections.* Stein † also has seen a case in which both organisms were demonstrated in fresh smears and by cultures from a resected cavity. In this case the pulmonary tuberculosis had preceded the coccidioidal infection. We did not observe this phenomenon in the seven patients with cavitary lesions.

SUMMARY

A historical review is given concerning the coccidioidal pulmonary residual and its relationship to the other phases of coccidioidomycosis.

Eleven cases of fibrocaseous granulomata of the lung due to Coccidioides immitis are presented in which surgical resection was performed.

The results of the coccidioidin, histoplasmin, and tuberculin skin tests are tabulated.

The results of the coccidioidal complement-fixation tests are tabulated, and the significance of the serologic reactions for coccidioidomycosis is presented.

The pathologic findings are described regarding the gross and microscopic characteristics of the four solid and the seven cavitary lesions.

The differential diagnosis is discussed regarding the etiology of focalized pulmonary granulomata of the fibrocaseous type.

The biologic significance of the coccidioidal pulmonary residual is discussed.

ADDENDUM

Recently F. S. Preuss, in a personal communication, reports that he has seen a focalized pulmonary granuloma of fibrocaseous type in which Blastomyces dermatitidis was established as the etiologic organism by culture. The small budding spherules characteristic of this organism were also demonstrated within the caseous center of the lesion in routine hematoxylin and eosin sections.

Dr. Charles E. Smith, University of California, School of Public Health, Berkeley, Calif., gave helpful suggestions.

Cmdr. Charles Hascall (MC), U.S.N., Pathology Service, United States Naval Hospital, San Diego, assisted in taking the photomicrographs, and William Von Allman, HM$_2$, U.S.N.R., Medical Photography Service, reproduced the chest x-rays and developed the photomicrographs.

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Reference 50, and Cotton, B. H.: Personal communication.

* Cotton, B. H.: Personal communication.

† Reference 49, and Stein, H. F.: Personal communication.

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Nephrotoxic Damage by Clostridium Perfringens Toxin

Protective Effect of Amino Acids Against Hyperglycemia and Renal Damage by C. Perfringens Toxin

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In recent years, a number of reports have appeared on the effect of various amino acids administered prior to a noxious agent in modifying or preventing the action of that agent. Large doses of a number of amino acids given prior to the administration of *dl*-serine in the rat have been reported to modify or prevent the effect of this nephrotoxic agent.[1] Various agents and chemicals have a preventative effect on organ changes when injected prior to a chemical which can produce physiologic or pathologic alterations. Sulfhydryl compounds administered in large doses prior to the injection of alloxan have prevented diabetes in rats.*

The production of acute pancreatitis and fatty liver by ethionine was prevented by the use of methionine.† Renal changes reported in feeding experiments have been aggravated by simultaneous feeding of cystine and prevented by methionine.‡ Mutually antagonistic phenomena have long been known to be manifested by naturally occurring amino acids through their effect on the formation or on the inhibition of the formation of proteins, or their effect on the roles carried out by proteins (enzymes, co-enzymes, surface sites, etc.) [10]

These reports have suggested an investigation of the effect of structurally different amino acids and other substances upon the nephrotoxic action and the experimental shock in dogs induced by intramuscular injections of Clostridium perfringens toxin.§ Since the action of the nephrotoxin was observed most strikingly in the disintegration of the mitochondria and in the pronounced and uniform reduction of the alkaline phosphatase activity in the brush border of the proximal convoluted tubules, a comparison of these effects, as well as of the physiologic and chemical alterations previously reported, would be useful as a method of study.

EXPERIMENTS

Nine groups of dogs were studied by use of the technical methods previously reported.‖ Group I consisted of 12 dogs. A lethal dose of Clostridium perfringens toxin¶ (8 ml. per kilogram of body weight) was injected intramuscularly into the midportion of the hind leg of the animals and massaged in order to increase the rate of diffusion of the toxin. It has been demonstrated previously that this is accompanied after four hours by the most diffuse and severe derangement of the mitochondria of the proximal convoluted tubules and the most uniform and severe destruction of the alkaline phosphatase activity.# Therefore, many of the animals were killed four hours after the injection of the toxin. Group II consisted of three animals who received only 3% *dl*-methionine intravenously (the pH of this solution, as well as the other solutions of amino

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* References 2-4.

† References 5 and 6.

‡ References 7-9.

§ References 11-14.

‖ References 13 and 14.

¶ The toxin was furnished by Eli Lilly & Company, Indianapolis.

References 13 and 14.

acids, was adjusted to 7.2). The dose of 1 gm. per kilogram body weight was employed in all of the experiments in which amino acids were administered and was of the same order of magnitude as has been previously reported by others.[1] After the intravenous injection, the animals were killed in four hours. Group III: Ten animals received *dl*-methionine followed within an hour by toxin and massage. While most of the animals were killed after four hours, some were killed at shorter or longer intervals. Group IV: Three dogs received only *l*(+)arginine monohydrochloride, while in Group V a like number also received *l*(+)arginine monohydrochloride followed by toxin and massage. Similarly, in Group VI three animals received only aminoacetic acid (glycine), and in Group VII three animals received aminoacetic acid followed by toxin and massage. In Group VIII the animals received 5% dextrose followed by toxin and massage, and in Group IX, isotonic saline followed by the injection of toxin and massage.

The following determinations were carried out on the animals at regular intervals: mean arterial blood pressure (readings made by the direct method from the femoral artery); urinary volume, glucose determination, and microscopic examination; hematocrit levels (Wintrobe method) and blood chloride,[15] urea nitrogen,[16] and glucose [17] determinations. The kidneys were removed quickly at the termination of the experiment, and sections of the kidneys, as well as other organs, were prepared by the freezing and dehydrating technique of Gersh and Bensley.* Other sections for special study were fixed in formaldehyde solution, N. F., diluted 1:10, Regaud's fluid, alcohol, and cold acetone for appropriate fixation.

RESULTS

Group I.—We have reported previously several observations following the effect of the injection of C. perfringens toxin in dogs, † and these will be summarized. Lethal doses of toxin injected intramuscularly produce extensive edema at the sites of injection and consequent hemoconcentration. This was accompanied by a statistically significant increase in the urea nitrogen and a reduction in the chloride of the blood, while the alkaline phosphatase and potassium remained unchanged. Although the blood pressure was not significantly altered in the first hour, there was a marked reduction in the volume of urinary output one hour after injection and abnormal urinary sediment (casts, erythrocytes, debris). The blood pressure readings revealed a gradual decline, reaching shock levels in four hours. In this group, two hours after injection gross blood was observed in the urine, with an occasional trace of sugar and increased amounts of protein; after four hours no urine was secreted. Abnormal phosphatase activity was not observed in the proximal convoluted tubules until close to four hours after injection of the toxin. At this time, the kidneys revealed a pronounced and uniform destruction of phosphatase activity (Fig. 1*B*), which was quite striking when compared with the normal (Fig. 1*A*). Histologic examination demonstrated a gradually increasing number of glomeruli showing vasoconstriction and swelling of the glomerular tuft, which was

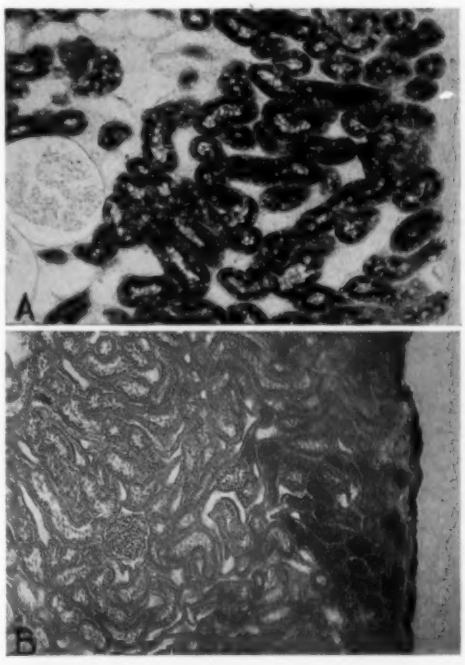


Fig. 1.—*A*, kidney from control animal, showing normal-appearing alkaline phosphatase activity in the proximal convoluted tubules; reduced about ⅔ from mag. × 200. *B*, kidney removed four hours after injection of toxin and massage, showing complete destruction of the alkaline phosphatase in the brush border of the proximal convoluted tubules; reduced about ⅔ from mag. × 200.

* References 18 and 19.

† References 13 and 14.

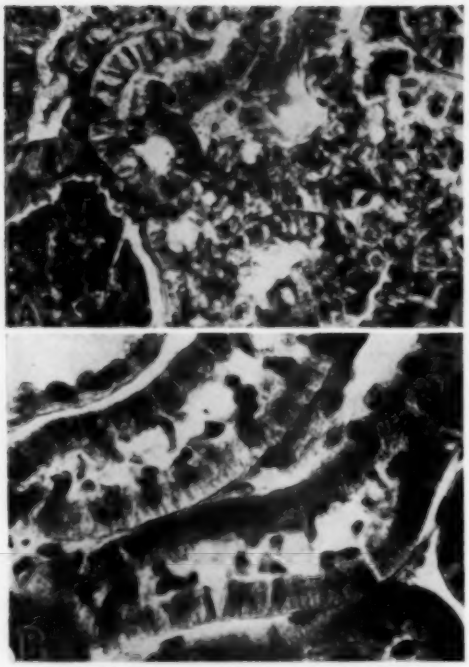


Fig. 2.—*A*, kidney removed four hours after injection of toxin and massage, showing severe and diffuse disintegration of the mitochondria in the proximal convoluted tubules; reduced about ⅔ from mag. × 800. *B*, kidney removed four hours after the administration of arginine, showing normal-appearing mitochondria in the proximal convoluted tubules; reduced about ⅔ from mag. × 800.

most pronounced between three and one-half and four hours after injection of toxin. There was a definite reduction in ferrocyanide filtration through the constricted capillaries. Between four and five hours after injection of toxin there was a wide dilatation of the glomerular capillaries, reduction in the size of the tufts, and generalized vascular congestion, which reflected the increasing circulating failure. With the wide dilatation of the glomerular capillaries, there was an increase in the glomerular filtration of ferrocyanide. In addition, hemoglobin was observed in Bowman's space and in some of the capillaries. Many of the proximal convoluted tubules presented a normal appearance, while others showed varying degrees of cloudy swelling, vacuolization, and disruption of cell walls. The distal convoluted tubules demonstrated a lesser degree of involvement; in many sections they differed little from the normal. The most striking observation was the severe and diffuse mitochondrial changes of the proximal convoluted tubules (Fig. 2*A*), showing varying degrees of disintegration and clumping to complete disappearance. Phenol red, which is secreted by the normal convoluted tubules and observed in the normal cells, was not found to be present.

A significant observation not previously reported was the occurrence of hyperglycemia, which reached its maximum two hours after injection of the toxin and was observed in all of the experiments. The peak of 152 ± 26 mg/100 ml. was almost twice that of the control level, 82 ± 7 mg/100 ml. ($t = 2.92, p < 0.05$) (Table).

Group II.—The effect of intravenous injection of a 3% solution of methionine on normal dogs was studied. There were no significant variations in the blood pressure, hematocrit value, urea nitrogen, and glucose, but there was a fall in the chlorides for the four-hour period of observation. There was an immediate and pronounced diuresis, the urinary volume in the first three hours being

Blood Glucose

	Blood Glucose, Mg./100 Ml.						
				Mean ± Standard Error			
Group	Control	After Injection 1 Hr.	After Toxin ½ Hr.	1 Hr.	2 Hr.	3 Hr.	4 Hr.
I. Toxin and massage	82 ± 7	.....	.....	94 ± 7	152 ± 26	128 ± 8	.....
II. Methionine	68 ± 1	65 ± 5	.....	.....	75 ± 5	69 ± 2	54
III. Methionine and toxin	85 ± 5	.....	.....	98 ± 21	73 ± 22	64 ± 18	83
IV. Arginine	77 ± 4	78 ± 3	.....	.....	59 ± 10	56 ± 20	114
V. Arginine and toxin	77 ± 7	.....	.....	81 ± 18	80 ± 20	70 ± 45	.....
VI. Aminoacetic acid	88 ± 12	121 ± 45	.....	.....	100 ± 23	79 ± 15	77 ± 11
VII. Aminoacetic acid and toxin	84 ± 3	.....	84 ± 2	72 ± 10	83 ± 14	64 ± 8	.....
VIII. Dextrose and toxin	111 ± 13	296 ± 14	104 ± 5	98 ± 2	95 ± 5	96 ± 13	105 ± 18
IX. Saline and toxin	95 ± 0.2	98 ± 1	133 ± 1	134 ± 14	176 ± 61	176 ± 56	165 ± 62

equal to the volume of fluid injected. The histologic sections of the various organs did not reveal any remarkable alterations.

Group III.—In this experiment the effect of 3% methionine administered one hour prior to the injection of toxin and massage was studied. The hourly blood pressure readings revealed a gradual drop in pressure, which reached shock levels at the end of four hours. If the control level is considered to be 100%, the average at the end of four hours was 53%. The hematocrit readings revealed a gradual but significant hemoconcentration, which reached a maximum in four to five hours. The blood urea nitrogen revealed a small but consistent rise after four hours, the control-period average was 11.1 ± 1 mg/100 ml., and after four hours it was 15.8 ± 2 mg/100 ml. This difference, however, was not statistically significant. The blood chlorides revealed a small decline which did not differ from that of the toxin-control animals. The blood glucose curve was flat, with a slight decline at the end of three hours (Table).

Urine output in five of nine animals showed no reduction in the first hour. In six of nine animals there was a marked reduction in the second hour, and in all of the animals there was a pronounced reduction in the third hour, the average being 2 ml., with a variation of 0 to 6 ml. The specimens were negative for sugar and revealed a trace of protein. There were numerous red cells, fewer white cells, and occasional casts. The number of cells was distinctly less than in the toxin-control animals.

Histologic examination of the kidneys revealed a widespread capillary dilatation in the glomerular tufts, which in some sections were small and in others were swollen, filling Bowman's space. The tubular structures were fairly well preserved and normal in appearance in the hematoxylin and eosin preparations. The mitochondria, while present, showed considerable fragmentation in some areas; in others, a fairly normal appearance. Compared with those of the toxin-control animals (Fig. 2*A*), the mitochondrial structures were better preserved in this group (Fig. 3*A*). There was a variable reduction of the alkaline phosphatase activity in the proximal convoluted tubules (Fig. 3*B*). Compared with the toxin control animals (Fig. 1*B*), there was evidence of the preservation of considerable phosphatase activity; however, there was not as much activity in the over-all picture as in the animals that received no toxin (Fig. 1*A*).

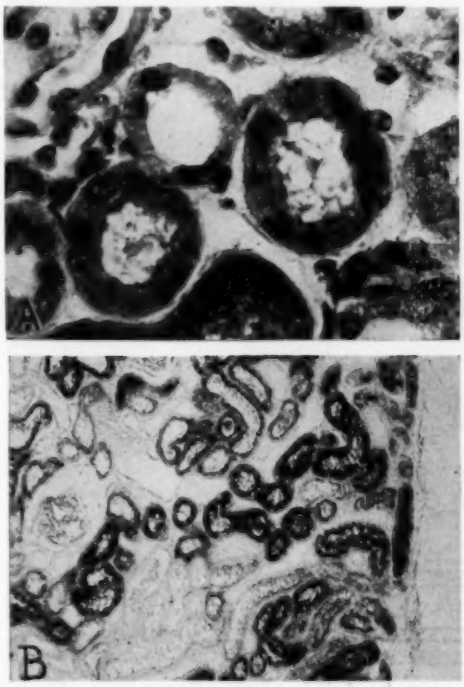


Fig. 3.—*A*, kidney removed four hours after injection of methionine and toxin, showing better-preserved mitochondrial structures; reduced about ⅔ from mag. × 800. *B*, same as Figure 3*A*, showing variable reduction of alkaline phosphatase activity in proximal convoluted tubules; reduced about ⅔ from mag. × 200.

Group IV.—In this group, the effect of the intravenous administration of arginine was studied. The blood pressure readings and the hematocrit values remained constant during a six-hour observation period. There was a variable increase in the urea nitrogen and a more uniform decrease in the blood chloride.

The urine excretion after injection revealed a pronounced diuresis for three hours, which was equal to 50% of the injected volume. The urine specimens were negative for sugar, protein, and abnormal urinary sediment.

The histologic sections of the various organs, including the mitochondria (Fig. 2*B*) and the alkaline phosphatase of the kidneys, were normal in appearance.

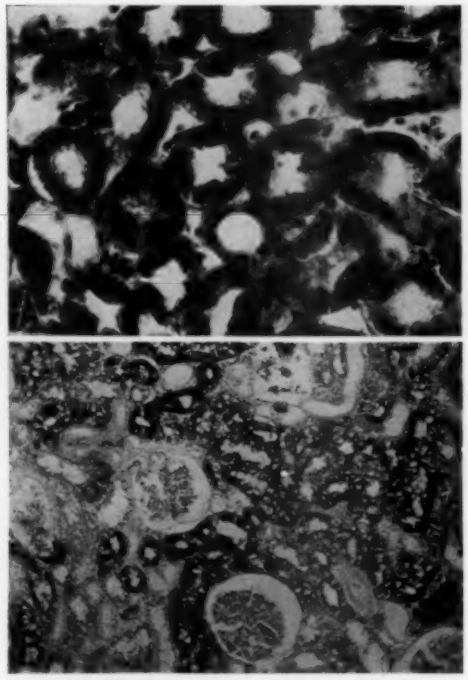


Fig. 4.—*A*, kidney removed four hours after the administration of arginine and toxin, showing good preservation of mitochondria; reduced about ⅔ from mag. × 700. *B*, same as Figure 4*A*, showing alkaline phosphatase in variable preservation and destruction; reduced about ⅔ from mag. × 200.

Group V.—In this group of three dogs, arginine was administered prior to the intramuscular injection of C. perfringens toxin. The blood pressure readings revealed a gradual drop, reaching shock levels in three hours, at which time the blood pressure was 51% of the control average. The hourly hematocrit readings reflected an increasing hemoconcentration. The blood urea nitrogen showed a small but consistent rise, which averaged 8 mg/100 ml. at the end of the experiment. The blood chlorides and blood sugar remained stable during the experimental period.

The urine output was large for the first two hours after the injection of the toxin, and during the third hour it dropped sharply as shock levels of blood pressure were reached.

Histologically, a number of tubules demonstrated derangement and disintegration of the mitochondria, and there were also a great many normal-appearing tubules (Fig. 4*A*). The number of tubules presenting mitochondrial changes was definitely less than in the animals receiving toxin alone. In comparison of the arginine group with the aminoacetic acid and the methionine groups, the first two revealed a very similar degree of mitochondrial preservation but a distinctly greater degree than in the methionine group. With respect to the alkaline phosphatase activity in the arginine group, a number of the proximal convoluted tubules demonstrated varying degrees of reduction of the alkaline phosphatase activity along the brush border, while many more tubules revealed an intense phosphatase activity (Fig. 4*B*). This observation differed sharply from that in animals that had received the toxin alone, but it was similar in many respects to that seen in the aminoacetic acid and methionine groups.

Group VI.—In this group, the effect of the intravenous administration of aminoacetic acid was observed, as a control study. The blood pressure readings revealed a slow and gradual decline of 35% at the end of a five-hour period of observation. The hourly hematocrit readings showed a slight rise. The blood urea nitrogen revealed a statistically significant elevation two hours after administration of aminoacetic acid, increasing from the control average of 11 ± 2.7 mg/100 ml. to 26 ± 0.5 mg/100 ml., which persisted until the end of the five-hour observation period. The blood chlorides at the same time revealed no statistically significant variations. While the blood sugar (Table) showed considerable variation from the control level

after the administration of aminoacetic acid, the mean levels did not differ significantly from the control. The urine volume revealed a diuresis lasting from two to three hours, and by the fourth hour it dropped sharply to the control level. The total volume of urine excreted during this period was one-half the volume of fluid administered. The urine contained no sugar, a trace of protein, and a number of erythrocytes and leucocytes.

Histologic examination of the various organs, such as the liver, kidneys, spleen, lungs, and muscles, revealed no alterations from the normal. The alkaline phosphatase activity of the liver and kidneys, and the mitochondria of the kidneys did not differ from the normal.

Group VII.—This group consisted of three animals to which aminoacetic acid was administered prior to the injection of C. perfringens toxin. These animals developed the usual edema at the site of the intramuscular injection of the toxin. The blood pressure dropped gradually to shock levels, and at the end of four hours the pressure averaged 50% of the control level. The hourly hematocrit readings revealed a gradual hemoconcentration. The blood chlorides demonstrated a slight drop over the four-hour interval, while the blood urea nitrogen revealed a distinct rise. The half-hourly and hourly blood sugar values (Table) remained at a fairly uniform level. The volume of urine excreted remained unaltered for two hours after the injection of the toxin, but during the third hour there was a marked reduction. This reduction in volume occurred at the time the blood pressure values approached or reached shock levels. The qualitative tests for sugar and protein in the urine were negative. Microscopically, only the terminal specimens showed erythrocytes and some casts.

Histologically, sections of the liver, spleen, and lungs demonstrated an acute passive congestion. In the injected muscles there was marked edema, which was seen in all the toxin-injected animals. In the kidneys, many of the glomeruli were swollen, filling Bowman's space, and there was also evidence of pronounced capillary constriction. The tubules revealed alterations similar to the toxin-control animals; the mitochondria, however, were more normal appearing than in the toxin-control animals. Figure 5*A* shows a number of normal-appearing tubules with dense rod-shaped mitochondria. The alkaline phosphatase presented a slight to severe degree of reduction in a number of tubules, and in the majority of tubules there was a qualitative intense phosphatase reaction (Fig. 5*B*). This was in sharp contrast to the almost complete destruction of alkaline phosphatase activity in the toxin-control animals.

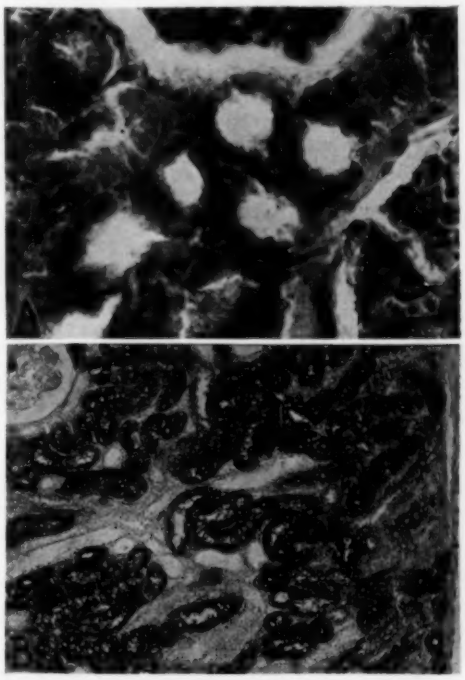


Fig. 5.—*A*, kidney removed four hours after administration of aminoacetic acid and toxin, showing better preservation of mitochondria as compared with after toxin alone (Fig. 2*A*); reduced about ⅔ from mag. × 700. *B*, same as Figure 5*A*, showing moderately intense phosphatase reaction; reduced about ⅔ from mag. × 200.

Group VIII.—This group consisted of three animals that received 5% dextrose in water, in the same volume-to-weight ratio as in the other experiments, prior to the injection of toxin. The hourly blood pressure

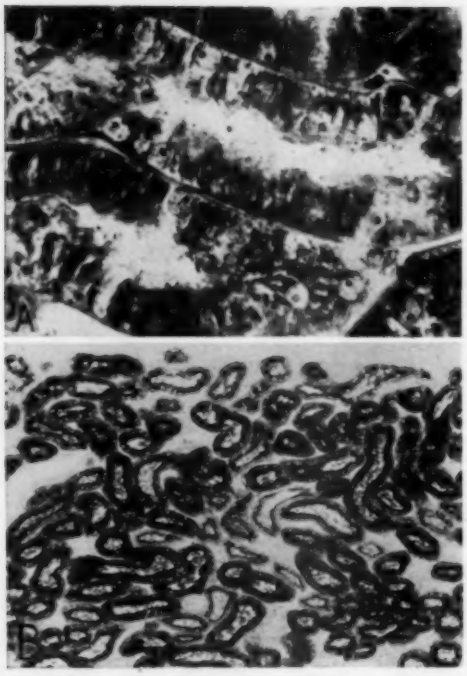


Fig. 6.—*A*, kidney removed four hours after the administration of dextrose and toxin, showing extensive destruction of the mitochondria; reduced about 2/5 from mag. × 800. *B*, same as Figure 6*A*, showing intense alkaline phosphatase preservation; reduced about 1/4 from mag. × 200.

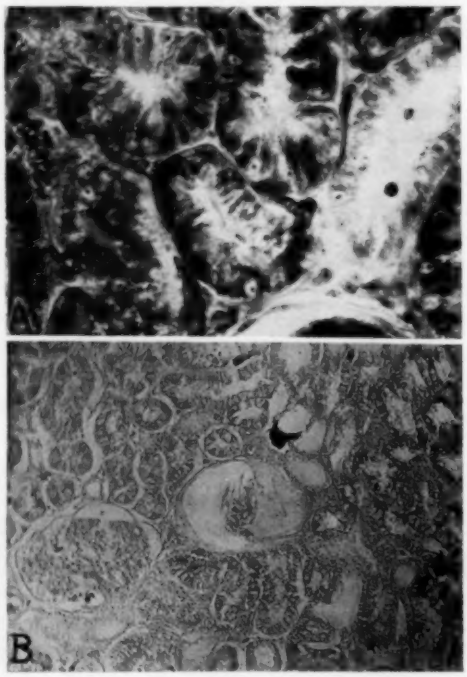
readings revealed a gradual drop to an average of 40% of the control level in four hours, at which time the animals were almost dead. The hematocrit readings demonstrated a gradually developing hemoconcentration. The blood urea nitrogen revealed a slight rise, while the blood chlorides remained constant. The blood sugar was elevated the first hour after the intravenous injection of dextrose; however, one-half hour after the injection of the toxin it returned to the preinjection level and remained relatively uniform to the end of the experiment (Table).

The urine volume demonstrated the expected diuresis, averaging 72 ml. for the first hour (or 18% of the injected volume of dextrose), and the first hour after the injection of the toxin it dropped sharply to an average of 7 ml. and decreased subsequently to 2 ml. per hour. In the first hour after injection of the toxin there was no glycosuria. Microscopically, all the specimens demonstrated numerous erythrocytes, occasional leucocytes, and amorphous material.

Histologically, the significant findings were observed in the mitochondria and in the alkaline phosphatase of the kidney. The mitochondria revealed widespread alterations, with varying degrees of disintegration and destruction (Figure 6*A*) similar to that seen in the animals receiving the toxin alone. The alkaline phosphatase revealed a varying degree of reduction in scattered tubules, and the majority of the tubules demonstrated very intense activity (Figure 6*B*).

Group IX.—This group of animals received 0.8% saline, in the same volume-to-weight ratio as in the other experiments, followed by the injection of toxin. The hourly blood pressure readings demonstrated a gradual drop over a four-hour period after the

Fig. 7.—*A*, kidney removed four hours after the administration of isotonic saline solution and toxin, showing severe destruction of the mitochondria; reduced about 2/5 from mag. × 800. *B*, same as Figure 7*A*, showing complete destruction of the alkaline phosphatase; reduced about 1/4 from mag. × 200.



injection of the toxin to a level of 40% of the control, at which time the animals were in profound shock. The hematocrit value was elevated, indicating a gradual hemoconcentration. There was a slight elevation in the blood urea nitrogen and little alteration in the blood chlorides. The blood sugar revealed a steady increase (Table), which in two hours after injection of the toxin reached a level which was statistically significant when compared with the control ($t = 22$, and $p < 0.01$).

The urine volume the first hour after the administration of saline averaged 25 ml., or 10% of the injected volume. The urine output one hour after the injection of the toxin dropped to an average of 4 ml. and then gradually diminished. There was no evidence of glycosuria. Microscopically, numerous erythrocytes were seen.

Histologically, there was widespread involvement, with varying degrees of disintegration and destruction of the mitochondria (Fig. 7*A*) similar to that observed in the animals receiving the toxin alone. Also, there was a widespread destruction of the alkaline phosphatase (Figure 7*B*) which was similar to that observed in the animals receiving the toxin alone.

COMMENT

The administration of large amounts of amino acids, as *dl*-methionine, *l*(+)arginine monohydrochloride, and aminoacetic acid, while producing marked immediate alterations in renal function, produced no demonstrable histologic alterations in the kidney, studies of which included the alkaline phosphatase activity and the mitochondrial structure. Experimental studies of renal function as performed by Pitts [20] revealed no evidence of chronic damage following large doses of amino acids; however, histologic findings were not discussed. In a study of renal reabsorption of methionine in normal dogs, Ferguson [21] has demonstrated that there is a high efficiency of the renal mechanism for the reabsorption of methionine. Considerable differences in the rate of reabsorption of various amino acids have been recorded by Pitts.[22] While the amounts resorbed stand in the decreasing order of aminoacetic acid, alanine, the amino acids of casein hydrolysates, and arginine, at all amounts filtered, it has been concluded that the large differences in their rates of reabsorption probably result from differences in their rates of combination with some cellular component common to the reabsorptive system for all.[22]

The mechanism of action of C. perfringens toxin injected intramuscularly or intravenously into dogs appears to be a complex one, and it probably represents a combination of factors: 1. There is a lecithinase component ‡ which appears to act on the phosphatides of cell surfaces, hemolyzing erythrocytes and affecting or destroying tissue cells. 2. The combination of a lecithinase and a hyaluronidase component § (plus small amounts of unknown toxin) may result in an edema-forming power. 3. There is the possibility that all the effects obtained are not due to the direct action of the toxin alone but may be ascribed in part to the products of tissue breakdown,‖ with the release of enzymes and toxic products and its effect on cellular permeability. Lethal doses of C. perfringens toxin injected intramuscularly followed by massage produced extensive edema at the site of injection, hemoconcentration, increased hemolysis, alteration in the electrolytes of the blood, a gradual drop in blood pressure to shock levels, a significant reduction in the volume of urine secreted that could not be correlated with the blood pressure, and alterations in the urinary constituents, as previously reported.¶

In addition, we have observed in our experiments that there was a diffuse destruction of the alkaline phosphatase of the brush border of the proximal convoluted tubules, a severe and diffuse intracellular disintegration of the mitochondria, and a failure of secretion of phenol red within the cells. There was an apparent correlation between the structural appearance of the mitochondria

‡ References 23 and 24.
§ References 25-27.
‖ References 27 and 28.
¶ References 13 and 14.

and the function of the tubules, as indicated by the secretion of phenol red.# Increasing the rate of diffusion of the toxin by massage resulted in the following changes: increased hemolysis, earlier appearance of abnormal urinary constituents, alterations in the pattern of development of intracellular * alterations in the tubules, and an increased rate and more severe and extensive destruction of the kidneys. The sequence of functional and structural alterations of both the glomeruli and the tubules which was observed suggested that the action of the toxin on the kidneys may be the result of these factors: (1) circulatory alterations and (2) the direct action of the toxin or toxic products on the cells.

Corcoran, Taylor, and Page,[29] in their report on acute toxic nephrosis based on a study of carbon tetrachloride poisoning, conclude that the essential lesion of toxic nephrosis is based on destruction of the epithelium of the nephron; its manifestations are much the same in spite of wide differences in the causative toxic agents.

It is of interest to note that Innes[30] in his study on renal cortical necrosis and other lesions in sheep produced by alloxan poisoning observed a resemblance of the clinical effect and pathology to that of "pulpy kidney" disease in lambs caused by C. welchii, Type D.

Ginzler[31] observed that in rabbits four days after the administration of a safranine O there developed selective necrosis of the renal tubular epithelium involving predominantly the proximal convoluted tubules; the glomeruli appeared uninjured, and there were minor degenerative changes and focal cellular necrobiosis in the loops of Henle and distal convoluted tubules. Some casts and necrotic material appeared in the collecting tubules. The deposition of a safranine in the kidneys has been reported by Sheehan,[32] and he observed that in unfixed frozen sections of the kidneys of rabbits killed within one minute after the injection of the dye, heavy staining of intermittent nephrons with the dye could be seen, whereas intervening ones might be altogether uncolored. Apparently some of the dye is secreted by the tubules from the peritubular capillaries whether there is interference with glomerular filtration or with renal circulation. An interesting comment made by Ginzler[31] is the similarity of renal injury resulting from various agents, such as mercuric chloride, chromates, uranium, bismuth, and, under certain conditions, cadmium. A similar histologic alteration in renal tubular injury following the injection of nephrotoxic agents has been reported with various organic compounds, and Ginzler is of the opinion that the nephrotoxic action of safranine, as well as that of other nephrotoxic organic compounds, parallels the action of heavy metals biochemically as well as pathologically and that these organic compounds exert their nephrotoxic effect by acting as inhibitors of vital enzyme systems.

Oliver and his associates,[33] in their studies on the pathogenesis of acute renal failure, stress the damage to the tubular epithelium and its relation to toxic and traumatic injuries with structural and functional alterations. According to these authors, two kinds of injury to the tubules in the kidney are encountered. One affects the proximal convoluted tubule and is mainly associated with nephrotoxic agents, and another involves random nephrons or any part of a nephron and is the result of a disruption of the renal tubule, and this is due to focal cortical ischemia (tubulorrhexis). In correlating anatomic and functional changes, Oliver believes that there is a unity in the pathogenesis of acute renal failure and that whether the lesion is nephrotoxic or tubulorrhexis, particularly in the human, is dependent upon the nature of the renal insult. However, in acute renal failure of nontoxic origin, such as shock, the insult is purely circulatory, and the term ischemuric episode is suggested for this complication. He integrates his analysis of this problem and comments on the unity of pathogenesis in the groups related to acute renal failure. Since there are many and sun-

References 13 and 14.
* References 13 and 14.

dry factors which may be associated with acute renal failure experimentally, as well as observed in human material, there is some clarification in the concept of the unity of the pathogenesis of this lesion, as shown by Oliver and his associates.

Of interest was the rapid development of hyperglycemia in our experiments here recorded, which reached a peak two hours after the injection of the toxin. It was observed in all the experiments, and, in most, the peak was about twice the control level. Associated with this hyperglycemia was an absence of glycosuria. Further detailed studies of this hyperglycemia will be reported subsequently. The hyperglycemia did not occur in the animals who received the amino acids (methionine, aminoacetic acid, or arginine) prior to the administration of toxin; nor in the animals who received dextrose. Hyperglycemia similar to that observed in the animals that had received the toxin alone was noted in the animals who had received saline solution, in the same volume as in the previous experiment, prior to the injection of toxin. Dilution alone of the effect of the toxin was apparently ineffective in preventing the hyperglycemia. Shifts of fluid and electrolytes of a large magnitude, with the development of hemoconcentration and local edema, were observed in the animals who had received the various solutions prior to the injection of the toxins. These alterations were similar to those seen in the group receiving the toxin alone. The similar action of structurally different amino acids and dextrose in blocking the hyper-amino-acetic-acid effect of C. perfringens toxin would appear to point to a relative lack of biologic specificity of the reaction. It would further suggest the probability that the amino acids and dextrose did not react with the toxin or toxic products to inactivate them chemically.

The group of animals who had received dextrose prior to the injection of toxin demonstrated the most intense and uniform alkaline phosphatase activity of the proximal convoluted tubules. In comparison with the normal animals, there were varying degrees of diminution in the alkaline phosphatase activity in a number of cells; in many others, no reduction was observed. In comparison with the diffuse destruction of the alkaline phosphatase activity in the toxin-control group, the difference was very striking.

The relative protective abilities of the various substances employed against the effect of C. perfringens toxin on the alkaline phosphatase, in decreasing order, were, first, dextrose; second, but considerably weaker, aminoacetic acid, and, third, arginine and methionine, which were about equal.

Glucose is absorbed in the first portions of the proximal convoluted tubule (Walker and Oliver),[34] the limitation in the resorptive process being the circumstance that the tubules are able to transfer only a certain maximal quantity of glucose from the tubules into the blood stream per unit of time (Shannon and Fisher).[35] Above this level, for example, glucose completely, reversibly, blocks the reabsorption of other sugars as xylose, which are reabsorbed by an identical process (Shannon).[36]

The quantity of dextrose employed in these experiments was below the maximal quantity that the tubules were able to transfer per unit of time. The considerable protection against a given dose of toxin by a given dose of dextrose could be partially overcome by increasing the rate of absorption of the toxin. In view of the fact that dextrose does not affect the action of the toxin in other portions of the kidney, nor the other widespread effects of the toxin, it is reasonable to assume that the protective action of the dextrose is not the result of any direct reaction between dextrose and toxin leading to an inactivation of the latter. The results indicate that the antagonism between dextrose and toxin with respect to alkaline phosphatase activity is possibly competitive in nature at the site of combination with this cellular component of the reabsorptive mechanism. Similarly, the competitive nature of the antagonism between dextrose and alloxan on the beta cells of the pancreas has been recently reported.[37]

The relative protective abilities of the amino acids employed against the destructive effect of the C. perfringens toxin on the mitochondria, in decreasing order, were as follows: Aminoacetic acid and arginine were of about the same degree of effectiveness, and methionine was considerably less effective. It has been pointed out that there are considerable differences in the rates of reabsorption of the various amino acids (Pitts) and that these probably result from the variable rates of combination with some cellular component common to the reabsorption system for all. The independence of the amino acid and glucose reabsorption mechanism has also been presented by Pitts.[22] In view of the fact that the amino acids do not inhibit most of the widespread physiological and biochemical actions of the toxin, it is reasonable to assume that the anticipated protective action of the amino acids is not the result of any direct reaction between the amino acids and the toxin leading to an inactivation of the latter. The results indicate that the antagonism between the amino acids and the toxin, with respect to the mitochondria, is possibly competitive in nature. The differences in the degree of protection are probably the result of the differences in their rates of combination with some cellular component in or associated with, the mitochondria, common to the reabsorption system for all.

Comparison of the protective effect of the amino acids on the alkaline phosphatase of the convoluted tubules with that of dextrose indicated both a greater intensity of the phosphatase activity and a greater uniformity of the reaction in the participating cells with the latter. In comparing the amino acids, it was seen that those that provided the best protection of the alkaline phosphatase also provided the best protection of the mitochondria. Dextrose, on the other hand, afforded the greatest protection of the alkaline phosphatase and no demonstrable protection of the mitochondria. These observations suggest the possibility of a different mechanism or mechanisms participating aside from, or in addition to, the mass action principle of Shannon[26] discussed above, which is seen most effectively at high plasma concentrations. The latter would be more applicable to the experiments with the amino acids than to those with dextrose.

The volume of urine secreted in the amino acid groups of animals was similar, with no reduction in volume for two hours after the administration of toxin. In the dextrose, saline, and toxin-control animals, the urine output showed a prompt reduction within one hour.

While the results indicate the possible competitive nature of the antagonism between dextrose and toxin, and between the amino acids and the toxin at these reaction sites, other factors, such as the effect on circulation and on cellular permeability, must be considered. The action of the amino acids or the dextrose did not appear to be one of combining with the toxin to block its enzymatic activity in the nature of an antibody-antitoxin (MacFarlane and Knight*) or to react with the toxin molecules. The reaction was different from that of lecithin, which combined with the lecithinase toxin molecule, as reported by Zamecnik and Lipmann.†

SUMMARY AND CONCLUSIONS

The effects of the administration of *dl*-methionine, *l*(+)arginine, aminoacetic acid, dextrose (5%), and saline upon action of Clostridium perfringens toxin in dogs are presented.

Lethal doses of C. perfringens toxin injected intramuscularly produce a hyperglycemia without glycosuria.

Injections of solutions of various amino acids and dextrose prior to the injection of toxin prevent the hyperglycemia. Injection of saline solution does not affect the formation of hyperglycemia.

The administration of dextrose prior to the toxin provides the greatest protection of all of the solutions employed for the alkaline phosphatase activity of the brush border of the cells of the proximal convoluted tubules,

* References 27 and 38.

† References 24 and 39.

with no apparent protection against the destructive effect on the mitochondria of these cells.

The administration of the amino acids prior to the toxin provides a lesser degree of protection for the alkaline phosphatase activity, in diminishing order as follows: Aminoacetic acid and arginine are of about the same degree of effectiveness, and methionine is less effective.

A definite protective effect of the amino acids against the destructive action of the toxin on the mitochondria of the proximal convoluted tubules is noted. The relative protective abilities of the amino acids is in the same order as for the alkaline phosphatase activity.

The administration of saline solution is ineffective in protecting against the destructive action of the toxin on the alkaline phosphatase activity and on the mitochondria.

Administration of the various solutions employed prior to the administration of toxin is ineffective against the lethal action, the physiological and biochemical alterations, and the histologic changes previously described.

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Primary Rhabdomyosarcoma of the Heart with Metastases

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A case of rhabdomyosarcoma arising in the right atrium is herewith reported. Approximately 350 primary tumors of the heart have been recorded in the literature of which 100 were classified as malignant.[13] Of these, only 46 were histologically identified as to type; they included 9 rhabdomyosarcomas, an incidence of 19.5%.* The current case is considered worthy of record because of the relative rarity of this type of tumor, the histologic documentation, and the masked clinical findings.

CLINICAL COURSE

A 64-year-old white man was admitted to the Veterans Administration Hospital, Bronx, N. Y., with a 3-week history of exertional dyspnea accompanied by a sensation of constant substernal pressure. Physical examination showed an obese, dyspneic man with distended cervical veins and a positive hepatojugular reflex. The blood pressure was 120/84. The lungs were clear. The heart sounds were within normal limits, with a regular sinus rhythm of 96 per minute. P2 was louder than A2. There were no murmurs heard. The liver edge was felt two fingerbreadths below the right costal margin, and there was right upper quadrant tenderness. The legs showed slight edema. Laboratory findings included a W. B. C. of 27,700 per cubic millimeter with a shift to the left. There was 1+ albuminuria. A roentgenogram of the chest showed some enlargement of the cardiac silhouette, with engorgement of the hilar and interstitial vessels and bilateral pleural effusion.

The patient was digitalized. On the third hospital day he became orthopneic and markedly tachypneic; the blood pressure fell and he became cyanotic. At that time, an electrocardiogram showed nonspecific ST and T wave changes with a rate of 140 per minute and auricular fibrillation. Shortly thereafter, the patient died. The cause of death was ascribed to cardiac failure due to heart disease of unknown etiology.

AUTOPSY FINDINGS

At necropsy, the pertinent findings were confined to the heart and lungs. There was a pericardial effusion consisting of 450 cc. of blood-tinged fluid. The parietal and visceral pericardial surfaces were studded with multiple firm, gray tumor nodules, the largest of which was 0.5 cm. in diameter. The heart weighed 450 gm.

The right auricular appendage was enlarged and measured 4 cm. in length. It was quite firm and the external surface was covered with a granular, fibrinous exudate. On section, the lumen was filled with a gray-white organized thrombus (Fig. 1). The myocardial border was indistinct. From the base of the appendage, a pedunculated protrusion extended through the atrium, prolapsing over the tricuspid valve. The surface of this projection was smooth, glistening, and continuous and blended with the atrial wall near the appendiceal opening. When transected, the interior of this lobulated mass appeared myxomatoid. At several points there were areas of fresh hemorrhage. The mass was so located that it resembled a ball-valve thrombus and could have interfered with the patency of the tricuspid valve. The

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Dr. Arthur C. Allen, Consultant Pathologist, Bronx Veterans Administration Hospital, assisted in establishing the diagnosis in this case.

* References 1, 3, 4, and 6 through 11.

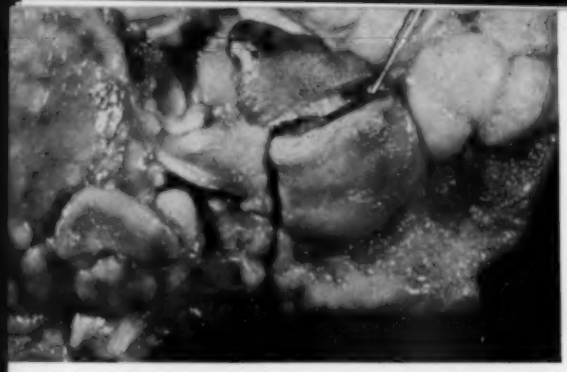


Fig. 1.—External view of right atrium showing tumor thrombus in appendage and pericardial reaction.

valve leaflets were thin, transparent, and delicate, and with retraction of the mass the edges could be reapproximated. The mitral, aortic, and pulmonic valves showed no gross abnormalities. The left atrium and the interatrial septum appeared normal. The wall of the right ventricle was 0.6 cm. thick, of which 0.4 cm. represented epicardial fat, while the left ventricular wall measured 1.8 cm. in thickness. The myocardium of both ventricles was of an even red-brown color and was firm throughout, showing no gross evidence of tumor or other abnormalities. The endocardium was smooth, thin, and translucent, except for the previously described areas in the right atrium. The circumferences of the valves were as follows: aortic 7 cm.; mitral 9 cm.; tricuspid 11 cm.; pulmonic 6.5 cm. The coronary arteries were normal in distribution and average in caliber, with occasional small atheromatous plaques in their walls. Their lumina were patent throughout. The aorta showed minimal atheromatous changes, which were more prominent in the abdominal portion. The main pulmonary arteries showed no evidence of tumor, thrombus, or other abnormalities. Examination of both lungs revealed several grayish-white metastatic tumor nodules in the subpleural parenchyma which varied in diameter from 0.5 to 2.5 cm. In addition, a focus of metastatic tumor of approximately 1.0 cm. surrounded a medium-sized artery in the hilar region of the right lower lobe (Fig. 2). The lumen of the vessel itself was occluded by a tumor thrombus. A small hilar lymph node showed tumor infiltration. No metastatic tumor or other pathologic changes of note were found in the vertebral bodies, ribs, and sternum. The central nervous system and skeletal musculature of the head and extremities were not examined.

Fig. 2.—Cut surface of right lung showing metastasis (probe).

Microscopic examination of sections of the right atrium showed a tumor composed of pleomorphic cells (Fig. 3). These include large strap-shaped cells containing longitudinal fibrils and occasional transverse striations, spindle or racquet-shaped cells, and large multinucleated cells with eosinophilic cytoplasm. Large round cells with radial striae and cells with a uniform, slightly granular cytoplasm were also found. The tumor cells blended almost imperceptibly with the myocardium of the atrial wall at many points and at one point extended to the epicardium. Adjacent myocardial fibers were compressed by the tumor, the edges of which extend into the cardiac muscle. The myocardial fibers, in contradistinction to the tumor cells, maintained their usual histological features and growth patterns. Striations which were seen with routinely stained sections (hematoxylin-eosin) were more distinct in sections stained with phosphotungstic acid hematoxylin. These were found in cells within the neoplasm at some distance from the interfaces of the tumor and cardiac muscle (Fig. 4). The location and morphology of these striated cells within the tumor mass militates against the probability that they represent myocardial fibers altered by the invading tumor.

Sections through the pedunculated atrial thrombus showed that its exposed surface was covered by endothelium; the matrix consists of an edematous, myxomatoid, relatively acellular stroma, throughout which were scattered spindle and strap-shaped tumor cells, as well as endothelial cells and fibroblasts. In addition, there were vessels and small areas of hemorrhage. The histological evidence suggested, therefore, that this mass represented tumor intimately associated with an organized thrombus.

Sections from grossly uninvolved portions of the heart showed no microscopic features of note.

Microscopic sections of the metastatic lesions in the lungs, hilar lymph node, and pericardium showed tumor histologically similar to that in the atrium. However, cross striations were not found in sections examined.

The final diagnoses were

1. Rhabdomyosarcoma of heart (right atrium) with metastases to lung, hilar lymph node, epicardium, and pericardium, with associated thrombosis of right auricular appendage
2. Hemorrhagic pleural and pericardial effusion
3. Tumor embolus in the lumen of a branch of the right pulmonary artery

COMMENT

Rhabdomyosarcomas vary greatly in histological appearance and in degree of differentiation. An absolute diagnosis depends upon finding rhabdomyoblasts, which may have three different characteristic shapes: (*a*) round, (*b*) strap-shaped, (*c*) racquet-shaped. All of these have an acidophilic cytoplasm and usually contain striations or longitudinal fibrils. The round cells may be small or large. Some are giant cells with marked peripheral vacuolation. In a number of these, processes extend from the nucleus into the cytoplasm between the vacuoles. The strap-shaped cells often contain groups of two or more nuclei arranged in tandem. Racquet-shaped cells have a nucleus at the broad expanded end, while the rest of the cell body tapers off. These three characteristic types may be associated with groups of pleomorphic cells, some of which may resemble poorly differentiated fibroblasts.

The tumor reported by us must be distinguished from the so-called rhabdomyomas of the heart. Two types of these tumors have been reported. One is accepted as a true benign neoplasm by Bigelow and co-workers,[2] who classify it as a fibromyoma resembling those found in the uterus. It is composed of fibrous tissue and primitive muscle elements. As reviewed in the work of Farber,[5] serious doubt has been cast upon the

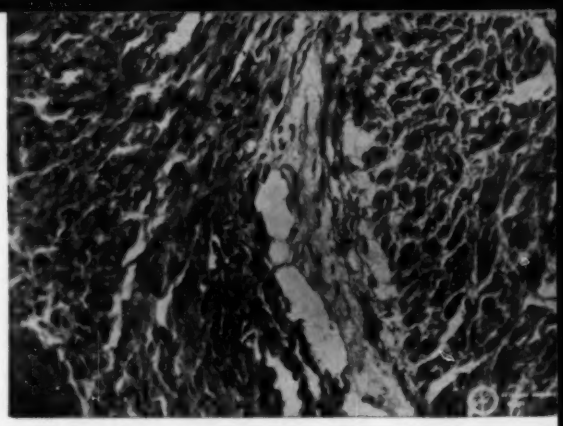
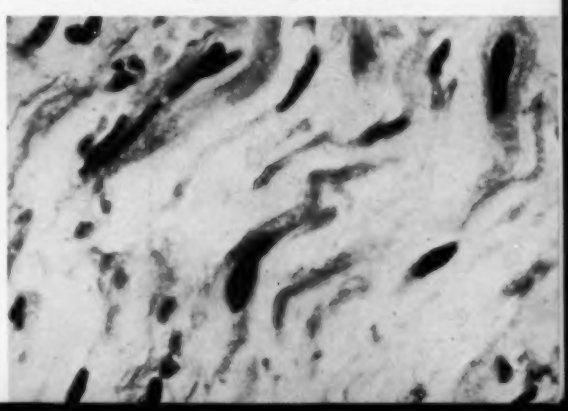


Fig. 3.—Right atrium showing junction of rhabdomyosarcoma (left) and cardiac muscle (right). Hematoxylin and eosin; reduced ⅓ from mag. × 430.

neoplastic nature of the other tumefaction previously classified as rhabdomyoma of the heart. This lesion shows a marked predilection for infants and is probably either hamartomatous or metabolic in origin (i. e., a localized form of von Gierke's disease). It usually consists of multiple sharply demarcated nodules composed of large cardiac fibers vacuolated with glycogen, the nuclei of which have delicate strands extending into the cytoplasm between vacuoles. The resemblance of these cells and their nuclei to the body and legs of a spider has led to their designation as "spider cells." This lesion is usually associated with other congenital anomalies and is most frequently found in cases of tuberous sclerosis.

The accuracy of the histological diagnoses of three of the nine previously reported cases of rhabdomyosarcoma, i. e., those of Bradley and Maxwell[3]; Larson and Sheppard,[7] and Muller,[10] has been questioned by Willis[14] because the tumor cells lacked definite cross striations. Five of these patients were female. The ages ranged from 14 to 75 years. Seven of them involved the atrial region; five on

Fig. 4.—Tumor cells showing striations, pleomorphism, and racquet-shaped cells. Hematoxylin and eosin; reduced ⅓ from mag. × 1200.



the right side. Two had polypoid projections into the atrial lumen. All nine showed pericardial alteration in the form of adhesions, tumor, or effusions. In only one case (Barnes and co-workers [1]) is involvement of skeletal muscle described, i. e., the deltoid. In six of these cases there were metastases. Metastases to the lungs were commonest (three cases). Also involved were the right hilus, lymph nodes, intestines, and pancreas. All showed marked histological pleomorphism.

The tumor reported by us arose in the right atrial wall of a 64-year-old man and metastasized to the lungs, hilar lymph node, epicardium, and pericardium. There were, in addition, a sanguineous pericardial effusion and a compromise of the atrial chamber by the polypoid projection of tumor into the right atrium. Clinically, the diagnosis was obscure, partially because of the rarity of this lesion, which in our case produced a clinical picture frequently seen in other much commoner types of heart disease. In addition, the patient's short hospital stay obviated a complete evaluation of his illness.

This case fulfils all the histological criteria for rhabdomyosarcoma. Although pleomorphic in character, many cells showed longitudinal fibrils in an eosinophilic cytoplasm, and characteristic strap cells and racquet cells were present. Some cells showed transverse cross striations. In view of the gross and histological considerations stated above, it appears clear that the myocardium was the site of origin.

SUMMARY

A case of rhabdomyosarcoma arising in the right atrium with metastases to the lung, pleura, hilar lymph nodes, and pericardium is reported. Nine cases previously reported in the literature are discussed, as well as the problem of classification and histological diagnosis.

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Granuloma of Lung Due to Radiographic Contrast Medium

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Whereas a search of medical literature reveals no previous cases of pulmonary reaction due to aspirated radiographic contrast medium, the following case, in which a distinctive reaction due to accidental aspiration of such medium was found, is reported.

CASE REPORT

A. M., an 81-year-old woman, was admitted to the Worcester City Hospital (Worcester, Mass.) because of dysphagia. Her past history revealed no association with any atmospheric condition of unusual dust content. Physical examination showed no significant deviation from the normal. Laryngoscopic examination showed a large tumor of the larynx obliterating all anatomical structures, biopsy specimen of which revealed an epidermoid carcinoma Grade 1. The patient had an upper gastrointestinal x-ray study, using the commercial preparation Gastropaque*; under fluoroscopic examination, it was observed that, although most of the medium passed readily into the stomach, some of it went directly into the lung fields (Fig. 1). This patient's condition gradually deteriorated, and she died 15 days after the x-ray study.

Autopsy findings (abstract) (Dr. Fite) revealed, in addition to the large tumor involving the larynx, multiple discrete nodules of firm grayish tissue averaging 5 to 10 mm. in diameter in the lower lobe of both lungs, most abundant in the left one. X-ray studies of portions of the lung tissue from autopsy showed numerous discrete radiopaque nodules averaging a few millimeters in diameter.

Microscopic examination of the lung tissue, including the nodules, noted on the x-ray

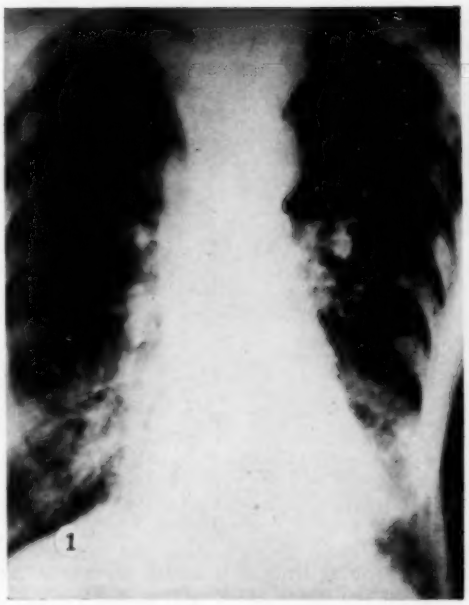
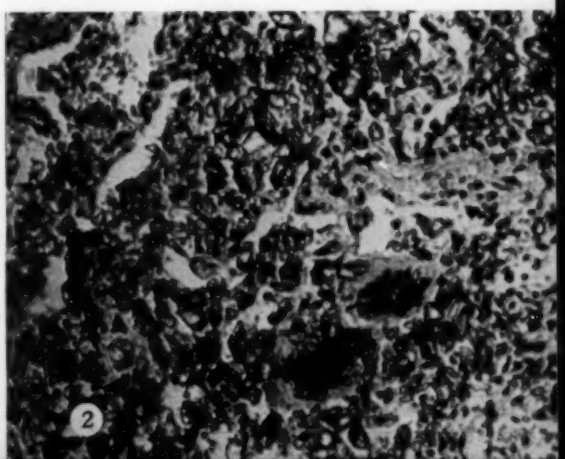


Fig. 1.—Case. Chest film showing nodular radiopacities in both lower lobes, most abundant on the left.

Fig. 2.—Case. Large numbers of crystals accompanied by macrophagic reaction are noticeable both in alveolar spaces and in interstitial tissue. Several large giant cells are present. Reduced 1/4 from mag. × 100.



From the Germantown Dispensary and Hospital and the Division of Pathology, Hahnemann Medical College.

*Manufactured by the Buck-X-Ograph Co., St. Louis.

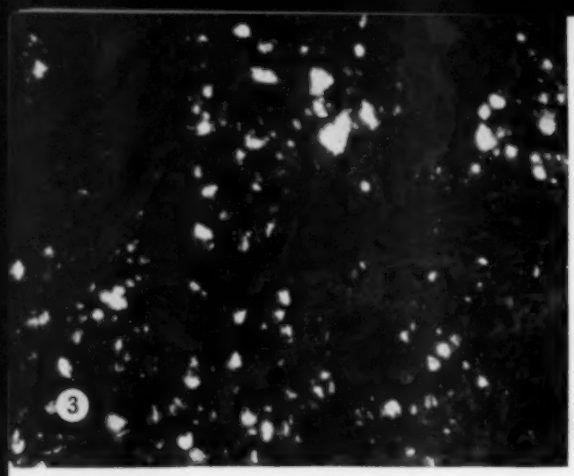


Fig. 3.—Case. Same field as Figure 2, polarized.

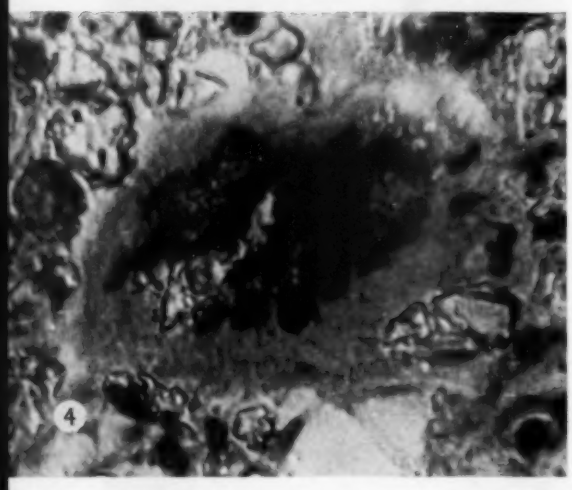
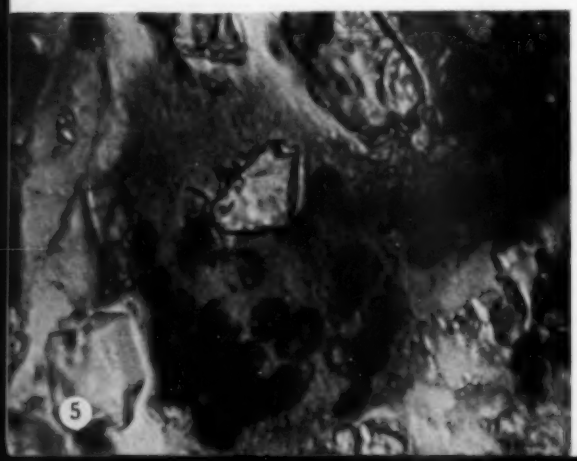


Fig. 4.—Case. Detail of giant cell seen in Figure 2, showing irregularly shaped crystals in the cytoplasm. Reduced 1/4 from mag. × 600.

study revealed a widespread filling of the alveolar spaces by large numbers of crystals of highly variable sizes and shapes. For the most part, they formed rhomboid polyhedral plates; frequently they were doubly refractile and refracted polarized light (Figs. 2 and 3). Together with these crystals there was intense accumulation of mononuclear macrophages, with numerous foreign-body-type giant cells; many of the macrophages, and practically all the giant cells seen, contained crystals in their cytoplasm (Figs. 4 and 5). An eosinophilic amorphous material suggesting coagulated protein was contained in the dilated alveolar spaces, and fibroblasts were imbedded in this material. A Van Gieson stain showed occasional delicate strands of collagen being laid down. The interstitial tissue was thickened by chronic inflammatory cell infiltration, and crystals were frequently seen in the septal tissue (Fig. 6). Frequently, the perivascular lymphatics were plugged by masses of small crystals accompanied by macrophages. Many areas of collapsed alveolar tissue were present in the involved areas. Occasional small isolated nodules were seen, composed of a central giant cell surrounded by macrophages. Sudan IV stain failed to show fatty material; a stain for acid-fast organisms and a MacCallum-Goodpasture stain for bacteria were both negative.

Fig. 5.—Case. Another giant cell containing crystals. Reduced 1/4 from mag. × 600.



A specimen of the lung tissue was submitted to a chemical laboratory for analysis.† The report of this analysis follows:

> Analysis has been made of dried pulmonary tissue. Since barium, if present, was presumed to be in the form of sulfate, the material was ashed to dull red heat, to reduce it to the mineral constituents. The major portion of the ash was then mixed with five times its weight of anhydrous sodium carbonate, and the mixture fused in a platinum crucible. After fusion, the residue was boiled in a small amount of water until thoroughly disintegrated, and filtered. The filtered residue was washed with several volumes of hot, normal sodium carbonate solution. Both the filtrate and the precipitate were retained.
>
> The collected precipitate was dissolved in dilute hydrochloric acid. Addition of dilute sulfuric acid gave a heavy precipitate, confirmed as barium sulfate, thereby confirming the presence of barium in the material.
>
> The collected filtrate was acidified with dilute hydrochloric acid and boiled to reduce the volume

† The chemical analysis was performed by Davis and Bennet, Inc., S. Gilbert Davis, Ph.D., Director.

and remove the carbonate. Addition of barium chloride produced a precipitate of barium sulfate, confirming the presence of sulfate in the material.

Spectrographic analysis of the portion of ash retained gave typical spectrum of barium of a density indicative of its presence in relatively large amount.

On the basis of these tests, it is indicated that barium salts in the form of sulfate is present in the portions of lung.

COMMENT

Granulomatous reactions to radiographic contrast material in other parts of the body have been previously reported. Numerous cases of peritoneal reaction due to barium sulfate, usually due to perforation of an ulcer during an upper gastrointestinal study, can be found in the medical literature.‡ A case of barium granuloma of the rectum has been reported by Beddoe, Kay, and Kaye.[5] A comparable reaction was found by us in a patient who, two weeks previously, had had a barium enema study; and in another case, in the bed of a duodenal ulcer, in a patient who had had an upper gastrointestinal series. The pulmonary reaction, as seen in our case, is entirely analogous with the intestinal and peritoneal lesions under comparable conditions, mainly consisting of an extensive accumulation of macrophages including crystals, giant cells, and the formation of collagenous tissue. Dissemination of barium to regional or remote lymph nodes has not been reported and could not be identified in our case either.

In an effort to verify the producibility of the reaction by the agent in question, we performed the following preliminary experiments. Five female rabbits, 6 to 8 months of age, were injected endotracheally with a watery suspension of Gastropaque; each animal received three injections of 1 cc. over a period of three days. Chest films were taken immediately before and after the injections, and the entrance of the material into the lungs was thereby verified. The rabbits were killed 15 days after the last injection, and autopsy showed discrete nodules of firm gray tissue, scattered at random in the lower lobes of the lungs. Microscopic examination of these nodules showed a reaction similar in all essen-

‡ References 1 through 4.

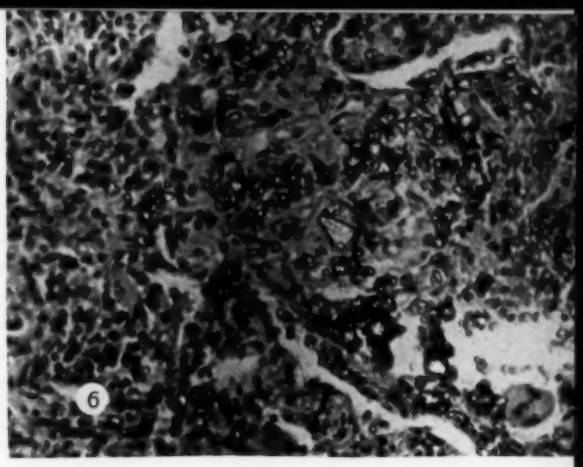


Fig. 6.—Case. Reactive fibrosis adjacent to crystals. Reduced 1/4 from mag. × 300.

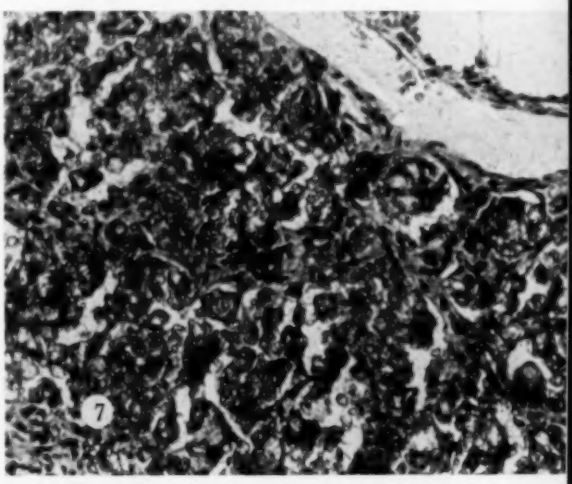
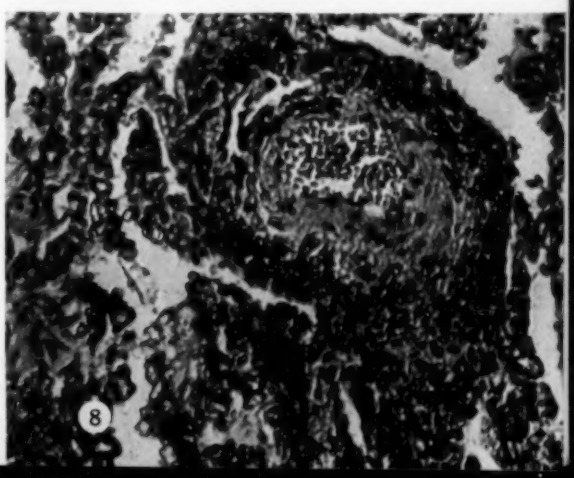


Fig. 7.—Rabbit lung, 15 days after Gastropaque injection. Alveolar spaces are filled with many macrophages containing crystals. A number of giant cells are present.

Fig. 8.—Rabbit lung, 15 days after Gastropaque injection, showing plugging of perivascular lymphatics by crystals and macrophages. Reduced 1/4 from mag. × 100.



tial aspects to that seen in our patient (Figs. 7 and 8). Crystals of the same appearance were abundant, and numerous macrophages and giant cells were present in their proximity; crystals and cells with the same characteristics were found in the perivascular lymphatics, and a beginning fibrosis was noted.

Huston, Wallach, and Cunningham [7] investigated the effect of barium sulfate endotracheally injected into the lungs of rats; they described four phases of tissue changes over a period of 120 days. They found giant cells appearing only after 90 days, and little or no fibrosis following the reaction. Our findings are at variance with theirs, giant cells being prominent in the reaction and a distinct, if minimal, collagen formation being present. This might be explained by the difference in species of animals used in the two experiments.

The exact component of the agent responsible for the reaction cannot be definitely identified. Gastropaque is described by its manufacturer as containing 97% barium sulfate (complying as to purity with the U. S. Pharmacopeia), a pentosan as a carrying medium, and a vanilla flavoring. The persistence of these crystals following routine formalin fixation and dehydration suggests that they are not pentosan crystals, which are water-soluble. Vanillin forms needle-like crystals. On the other hand, barium sulfate forms rhomboid plate crystals, identical in appearance to those seen in the tissues described here. Although it is likely that the granulomatous reaction was elicited by the barium sulfate, the exact nature of the agent is being investigated further in controlled experiments.

SUMMARY

A case is reported of barium granuloma of the lung, following accidental aspiration of radiographic contrast medium. The lesion is identical to comparable lesions observed in the alimentary tract by others and by me. Endotracheal injection of barium in rabbits has resulted in the production of granulomas comparable in all respects to that found in the case here presented.

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The Transplacental Diffusion of Carbon Monoxide

A Review and Experimental Study

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A. J. McBAY, Ph.D.
and
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Conflicting reports have appeared in the literature concerning the passage of CO across the placenta from maternal to fetal blood and its subsequent effect on the fetus. As recently as 1950, two cases were reported in which pregnant women succumbed to CO poisoning, with high saturation of the maternal blood with the gas but with no CO detectable in the fetal blood.[10] It was concluded that the gas does not pass from the mother to the fetus. Recently one of us (R. F.) was consulted concerning a case of CO poisoning of a woman near term.* The mother survived, but the child was born dead. Examination of the fetal blood † showed a high saturation with CO. This confusion led to an extensive review of the literature and animal experimentation to clarify the above observations.

On review of the literature, we were able to find and study thoroughly 20 cases of CO poisoning during pregnancy, which are summarized in Table 1. In eight of these cases ‡ the mother died of CO poisoning and the fetus was found dead in utero. In six of the cases § the mother survived the poisoning and the baby was born dead. In the remaining six cases || both mother and fetus survived. In addition to this group of 20 cases, Desclaux and co-workers [7] cite 3 cases in which there was fetal and maternal survival, but the original reports are not accessible to us at this time.

Review of these cases shows that the evidence for CO poisoning of the mother and the effect on the fetus varies greatly. CO levels are not reported for either fetal or maternal blood in 10 of the cases, and the evidence of poisoning is based wholly on clinical history and observation, which, for the most part, is clear, concise, and classic. In the other 10 cases, CO determinations of the fetal blood, viscera, or pleural fluid were made. In seven of these cases both mother and fetus were found dead and CO levels were determined for both. In three of these cases ¶ CO saturations of the maternal blood were 48%, 88%, and 65% and no CO was detected in the fetal blood. These workers concluded that CO does not pass from maternal to fetal blood. However, in four of the remaining cases of this group CO was detected in the fetus, and in two of these CO-saturation values of the fetal bloods were one-seventh and one-third those of the maternal blood.# In the other two cases the liver only was analyzed, and CO was de-

From the Department of Legal Medicine, Harvard Medical School (Miss Algeri, Dr. McBay, and Dr. Ford); from the Department of Pathology, Harvard Medical School (Dr. Curtis).

* Muller, G. L., and Graham, S.: To be published.

† Stratton, F.: Personal communication to the authors.

‡ References 2, 4, 6, 13, and 19.

§ References 4, 11, 28, 30, and 31 and Muller, G. L., and Graham, S.: To be published.

|| References 3, 4, 7, 18, 20, and 34.

¶ References 13 and 19.

References 2 and 6.

tected and found to be one-third the amount found in the mother.[6] In the remaining 3 of the 10 cases in which determinations were made, the mother survived the poisoning. The fetuses were born dead, and in two of these determinations were reported for the fetal bloods.* In the other case, a qualitative determination was done on pleural fluid only, which was definitely positive for CO. Hence, in summary of the cases appearing in the literature, it is concluded that the majority report the actual passage of CO from the maternal to fetal circulation, there being only three instances where this is considered not to have occurred.

* Reference 31 and Muller, G. L., and Graham, S.: To be published.

Throughout the years, workers have exposed pregnant animals to CO, and their results can be used to explain these confusing clinical observations. The first work was done in 1877 by Högyes,[14] who reported that when two pregnant rabbits were killed by the introduction of CO for one and one-half and two and one-half minutes through a tracheal catheter no CO was detected in the fetal circulation by spectroscopic methods. He concluded that CO did not cross the placental barrier. Fehling,[10] in the same year, arrived at a different conclusion. He showed that when pregnant rabbits were exposed to illuminating gas for more than one hour CO could be detected in the fetal blood. Fehling placed two living fish in a vessel

TABLE 1.—*Human Cases*

Mother			Fetus			
Mo. Preg. at Time of Exposure	State	Blood CO	State	Blood CO	Remarks	Reported by
Full term	Dead	No determ.	Dead	No determ.	Gas-filled room	Breslau 1859 [4]
8	Alive	No determ.	Stillborn	No determ.	Both women in same room, accidental	Breslau 1859 [4]
9	Alive	No determ.	Alive	No determ.		Breslau 1859 [4]
7-8	Alive	No determ.	Stillborn	No determ.	Accidental poisoning; macerated fetus	Freund 1859 [11]
Near term	Alive	No determ.	Stillborn	4.9 vol. %	Attempted suicide	Tissier 1909 [21]
8	Dead	16.9 vol. % (60% saturation)	Dead	4.6 vol. % (18% saturation)	Accidental; woman apparently suffering from systemic disease; history poor	Balthazard & Nicloux 1913 [2]
6	Alive	No determ.	Stillborn	+ CO chest fluid	Accidental; husband and child in same room; child died, husband survived	Phillips 1924 [23]
Near term	Alive	No determ.	Lived 9 days	No determ.	Attempted suicide; fetal brain injury with classic basal ganglia lesions	Maresch 1929 [18]
8	Alive	No determ.	Lived 6 days	No determ.	Fetal brain injury; cerebral edema, classic basal ganglia lesions described	Neuburger 1935 [20]
4	Alive	No determ.	Alive	No determ.	Child at time of report 4 yr. old with brain damage since birth	Brander 1940 [3]
9	Dead	65% saturation	Dead	Neg. for CO	Suicide, gas-filled room	Helpern & Strassman 1943 [13]
3	Alive	No determ.	Died after 8 months	No determ.	Accidental	Zourbas 1947 [24]
8	Dead	15.3 vol. %	Dead	2.2 vol. %	Not known if accidental	Dérobert, Le Breton, & Bardon 1949 [6]
2	Dead	66% saturation (liver)	Dead	22% (liver)	Suicide	
3	Dead	66% saturation	Dead	5 vol. % (liver) Macerated	Suicide	
9	Dead	88% saturation	Dead	Neg. for CO	Suicide	Martland & Martland 1950 [19]
5	Dead	48% saturation	Dead	Neg. for CO	Accidental	
Near term	Alive	No determ.	Stillborn	49% saturation	Accidental	Muller 1950 *
5	Alive	No determ.	Alive	No determ.	Accidental; husband in same room, comatose, survived; child 8 yr. at time of report with idiocy, etc.; family normal	Desclaux, Soulairac, & Morlon 1951 [7]
?	Alive	No determ.	Dead	No determ.	Aborted, accidental	Seifert 1952 [30]

* Muller, G. L., and Graham, S.: To be published.

containing diluted ox-blood saturated with CO and found that when the fish died, three hours later, CO could be detected in the fish blood. He concluded that CO dissociated from the carboxyhemoglobin and diffused through the branchi of the fish and into the fish blood to combine with the hemoglobin present there. He considered this similar to the passage of CO across the villus from maternal to fetal blood. Gréhant and Quinquaud [12] showed that upon exposing pregnant dogs to CO for 35 minutes CO appeared in the fetal blood and was about one-sixth that found in the maternal circulation. Dreser [9] in 1891 exposed a pregnant rabbit intermittently to CO gas for 35 to 40 minutes and, by determining the oxyhemoglobin, found that the fetal bloods were about one-half as much saturated with CO as the maternal blood. The work of Nicloux,† begun in 1901, concerning the passage of CO from maternal to fetal blood, constitutes a most outstanding contribution to this study up to the present time. He demonstrated that, upon exposure of pregnant guinea pigs to low concentrations of CO in a mixture of CO and air for long periods of time, the degree of saturation of fetal blood was the same as that of the maternal blood.[21] Using a very high concentration of CO in the CO-air mixture, he demonstrated that the mother succumbed quickly, with a high degree of saturation of her blood with CO and a low saturation of fetal blood with CO. His work does not include the saturation of the blood of each fetus with CO nor does he describe the condition of the fetuses. Williams and Smith,[23] in 1935, exposed pregnant rats to illuminating gas daily and found that litters of inferior rats were born, which usually died shortly after birth. On prolonged exposure of the pregnant rats to illuminating gas the offspring were born dead, and after successive exposure of a rat over several pregnancies apparent sterility ensued. Dérobert and co-workers,[6] in 1949, were interested in the CO content of fetal organs as well as the blood after exposure of the pregnant rabbit to CO. They showed that the amount of CO passing from the maternal to the fetal blood depends upon the duration of exposure of the mother to the gas. Seifert,[30] in 1952, investigating the rapidity with which CO passes from maternal to fetal blood, found that two pregnant rabbits exposed to CO for one and three-quarters hours died and that the CO saturation of the fetal bloods was the same as that of the mother. When the mother was exposed daily from the 20th gestational day on to low concentrations of CO and a blood saturation of 30% was reached, 9 living, active, apparently normal fetuses were removed at term. The maternal blood was negative for CO, and the fetal bloods were 3% saturated. Another rabbit was treated in a similar manner, starting with the 15th gestational day, and at term three living and five dead fetuses were delivered. The maternal blood was negative for CO, and the fetal bloods were 3% saturated.

From this review of the literature, it is conclusively shown that CO does pass from the maternal to the fetal circulation. Facts from isolated animal experiments reported over the past 77 years indicate that the percentage of saturation of fetal blood with CO varies directly with the time of exposure of the mother to an atmosphere containing CO and also with the concentration of CO in that atmosphere. In summary of the clinical cases reported, the following situations can be found when a pregnant woman is exposed to CO gas: 1. Both the mother and fetus die. 2. The mother survives, and the fetus dies. 3. Both mother and fetus survive the poisoning. The percentage of saturation of fetal blood varies from case to case, and various conclusions have been drawn concerning the diffusion of CO across the placental barrier and the mechanism of death of the fetus. Hence, animal experiments were planned to simulate all the conditions of exposure in the clinical cases reported. Through the years, the methods for the determination of CO have varied greatly, and in the early work—as well as in some of the recent work

† References 5 and 21-25.

—qualitative methods were used. Repetition of some of the animal work with use of an accurate quantitative method for the estimation of carboxyhemoglobin seemed advisable in order to clear up some of the discrepancies existing in the interpretation of the experimental work.

EXPERIMENTAL PROCEDURE

The animal used was the rabbit, since it breeds readily and has a short gestational period, from 30 to 31 days. It was shown by Anson and coworkers,[1] who studied the spectra of hemoglobins of different species, that the affinity for CO and O_2 varies greatly, and this is true when man and the rabbit are compared. However, in experimental studies of the effect of CO poisoning during pregnancy the rabbit, which has been the animal of choice, the dog, the rat, and the guinea pig have all shown similar results, which correlate closely with those seen in the human.

The pregnant animal was exposed to illuminating gas ‡ in an air-tight box measuring 24 × 11 × 11 in. (60 × 27 × 27 cm.). Illuminating gas entered the chamber through an inlet in the bottom. An attempt was made to maintain a certain CO saturation of maternal hemoglobin over a fixed period of time. The condition of the rabbit was observed throughout the entire exposure period, and the animal was removed for short periods in order to obtain blood samples.

The method used for the determination of CO was the spectrophotometric procedure as outlined by Hunter,[16] with some modification. The method is rapid and accurate with amounts of blood as small as 0.02 ml., and the results agree favorably with those obtained by the Van Slyke and Salvesen procedure.[32]

About 0.02 ml. of blood was placed directly into 5 ml. of 0.1% sodium carbonate solution. Determinations were carried out immediately, since it was found that losses may occur with a lapse of time. The blood sample was diluted when necessary and transferred to a 1 cm. cuvette. A 10 mg. portion of sodium hydrosulfite was added to reduce the oxyhemoglobin. A comparison cell containing 0.1% sodium carbonate was prepared. Absorbances at 470 and 600 mμ were determined with a Beckman DU spectrophotometer. The percent saturation of the hemoglobin with CO was read from prepared nomographs. Control determinations of blood and tissue of macerated fetuses not exposed to CO in utero were made. CO was not detected.

‡ The composition of the illuminating gas was as follows: CO, 6.8%; CO_2, 2.3%; O_2, 1.6%; CH_4, 24.3%; C_2H_4, 2.4%; H_2, 47.8%; N_2, 11.6%; illuminants, 3.2%.

Thirteen pregnant rabbits were exposed to illuminating gas under conditions which fall into three definite categories: (1) acute exposure of the pregnant rabbit to high concentrations of illuminating gas, (2) interval exposure of the pregnant rabbit to illuminating gas at varying concentrations in the mixture inspired, and (3) chronic exposure of the pregnant rabbit for several long periods over several days to low concentrations of illuminating gas. The pregnant animal would sometimes live through the exposure and at other times die. If the mother died, she was autopsied immediately and CO saturations of her blood and the blood of each fetus were determined before normal regular respiratory movements of the fetuses had commenced or while the first few irregular respiratory movements were taking place. If the mother survived the exposure and did not deliver, a hysterotomy was performed on the following day and the fetuses were handled as described above. The day following the last exposure was chosen for hysterotomy, as the fetuses, should they be dead, would show postmortem changes indicative of death due to the exposure to illuminating gas and not to factors involved in operating on the pregnant animal. Hysterotomy was chosen rather than normal delivery, since the mother may devour the stillborn fetuses and in some cases, when disturbed during pregnancy, the living fetuses.

RESULTS

I. Exposure to Illuminating Gas: Death of the Pregnant Rabbit

A. Acute Exposure.—Experiments 1 and 2 were designed to study the fetal mortality and fetal CO levels when the pregnant rabbit was rapidly killed by exposure to large amounts of illuminating gas.

Experiment 1.—A rabbit at term was exposed to a high concentration of illuminating gas for a period of five minutes, at the end of which time she convulsed and died. At the time of death the maternal hemoglobin was 84% saturated with carbon monoxide. The eight fetuses were alive and active, and their hemoglobins were from 11% to 16% saturated with CO.

Experiment 2.—A rabbit at term was exposed to illuminating gas at a lower concentration. She convulsed and died within 15 minutes, and her hemoglobin was 82% saturated with carbon monoxide. Six fetuses were removed. Five of them were alive and active, and hemoglobin saturations with CO varied from 17% to 24%. One was dead, and the hemoglobin was 25% saturated with carbon monoxide.

TABLE 2.—*Exposure of a Pregnant Rabbit to Illuminating Gas Over Short Intervals: Maternal and Fetal Death*

Mother				Fetuses *	
Exposure, Min.	Blood CO,† %	In Air, Min.	Blood CO on Return to Chamber	Wt., Gm.	Blood CO, %
10	78	60	21	44	31
15	93	60	19	39	67
11	72	30	24	48	61
20	57	120	0	34	49
3	57	Died		41	57
				35	50
				30	40
				28	27
				26	40
				37	58
				53	57
				48	50

* The fetuses are listed in the order of removal from the uterus in every table.
† Throughout this paper the blood CO refers to the per cent saturation of hemoglobin with CO.

Hence when a pregnant rabbit is exposed to large amounts of illuminating gas and dies within 5 to 15 minutes the majority of fetuses are born alive and active and the hemoglobin saturation values are one-eighth to one-third that of the maternal hemoglobin at death. The average value of carbon monoxide saturation of fetal hemoglobin is slightly higher in the fetuses born of the mother exposed for 15 minutes than in those of the mother exposed for 5 minutes.

B. Exposure over Short Intervals.—The following two experiments were designed to study fetal mortality after intermittent exposure of the mother to large amounts of illuminating gas over a longer period of time than in the acute exposure.

EXPERIMENT 3.—A 28-day-pregnant rabbit was exposed for periods of 10 to 20 minutes to illuminating gas, as shown in Table 2. In the fifth period of exposure the animals lapsed into coma and died. Twelve dead nonmacerated fetuses were found. The percent saturation of hemoglobin with CO varied markedly in the individual fetuses, as seen in Table 2.

EXPERIMENT 4.—A rabbit similar to that used in Experiment 3 was exposed to about the same quantity of illuminating gas except that the intervals of exposure were on two successive days. Ten dead nonmacerated fetuses were removed, and the results were similar to those in Experiment 3. The variation of carbon monoxide saturation of hemoglobin in individual fetuses was as marked as in Experiment 3.

These two experiments, in contrast to those under acute exposure, show that if exposure of the pregnant animal to illuminating gas occurs over a long period of time the entire litter will be born dead and the percent saturation of their hemoglobin will be much greater than in the acute exposure experiment and that it will in some instances approach the maternal level.

C. Continuous Exposure over a Long Interval.—This experiment was designed to study fetal mortality and CO blood levels of the fetuses when the pregnant rabbit was exposed to illuminating gas continually over a long period of time, finally dying.

EXPERIMENT 5.—A 28-day-pregnant rabbit was exposed to illuminating gas continually over a period of 60 minutes. The entire litter of six fetuses was dead, and the percent saturation of the fetal hemoglobins with CO approached the highest maternal values, as seen in Table 3.

II. EXPOSURE TO ILLUMINATING GAS: SURVIVAL OF THE PREGNANT RABBIT

In this series of experiments every effort was made to keep the animal alive, although conditions of exposure were similar to those in which the animal died. This was accomplished by using lower concentrations of illuminating gas and by removing the animal from the chamber at the first sign of convulsions or coma. Marked respiratory distress was a common feature indicating severe anoxia of the mother.

A. Acute Exposure.—These experiments are similar to Experiments 1 and 2, except

TABLE 3.—*Long-Interval Exposure of a Pregnant Rabbit to Illuminating Gas: Maternal and Fetal Death*

Mother		Fetuses	
Exposure, Min.	Blood CO, %	Wt., Gm.	Blood CO, %
9	47	40	63
28	60	41	50
39	72	36	37
54	67	39	49
61	63	29	65
		28	51

that the pregnant rabbit survived exposure to illuminating gas in amounts large enough to cause convulsions and loss of consciousness.

EXPERIMENT 6.—A 27-day-pregnant rabbit was exposed to large amounts of illuminating gas for periods of two, three, five, and three minutes. At the end of the first period the animal convulsed, and at the end of each successive period the animal became drowsy. The periods were separated by 3 minutes except the third and fourth periods, which were separated by 10 minutes. The mother's hemoglobin was from 63% to 77% saturated with carbon monoxide from the first period to the end of the third period, an interval of 16 minutes. The mother survived the exposure, and the entire litter of eight living active fetuses was removed the following day. CO was undetectable in any of the fetal blood.

EXPERIMENT 7.—A 28-day-pregnant rabbit was treated under conditions similar to those of Experiment 6, but the exposure time was over a thirty-minute period and the animal was removed from the chamber only long enough for the taking of blood samples. The saturation of the maternal hemoglobin with carbon monoxide over this period varied from 63% to 69%. Five living active fetuses were removed the day after the exposure, and, as in Experiment 6, CO was undetected in any of the fetal bloods.

It is shown by these two experiments that a pregnant rabbit can be exposed to amounts of illuminating gas large enough to cause convulsions and severe maternal anoxia and yet survive and apparently normal active fetuses be delivered.

B. Exposure over Several Short Intervals.—The following three experiments are similar to Experiments 3 and 4; however, the mother survived the exposures. The experiments consist of exposing pregnant animals to large amounts of illuminating gas for two 6-minute intervals, the intervals being separated by 40 minutes in air.

EXPERIMENT 8.—A 28-day-pregnant animal's hemoglobin was 61% saturated with CO after the first six-minute exposure period and 70% saturated with CO after the second six-minute exposure period. Throughout the entire exposure periods the animal appeared drowsy but never became comatose or convulsed. The following day 10 fetuses were removed. Eight were living, active, and negative for carbon monoxide. Two were dead and macerated, and the hemoglobin of each was 40% saturated with CO.

EXPERIMENT 9.—A 28-day-pregnant animal's hemoglobin was 66% saturated with CO after both of the six-minute exposure periods, and it behaved throughout the entire exposure period in the same manner as the rabbit in Experiment 8. The day after exposure, six normal active fetuses were removed, and the bloods of all were negative for CO.

EXPERIMENT 10.—A 29-day-pregnant rabbit was exposed under exactly the same conditions as the other two. However, just before the first six-minute exposure period she delivered one normal active fetus. The maternal hemoglobin was 69% saturated after the first six-minute period and 75% saturated after the second six-minute period of exposure. Two hours after the last exposure period she delivered 13 fetuses. Nine of these fetuses were active and apparently normal, and the saturation of their hemoglobin with CO varied from 13% to 23%. Two fetuses were dead and nonmacerated, and their hemoglobin was 19% and 25% saturated with carbon monoxide. One fetus had been decapitated by the mother, and its hemoglobin was 12% saturated with carbon monoxide. The remaining fetus was extremely macerated, and its blood was negative for CO, indicating death days before the exposure.

These rabbits were treated very similarly except that the highest percent saturation of the hemoglobin of the pregnant rabbit in Experiment 9 was 4% lower than the highest maternal level in Experiment 8 and 9% lower than that attained in Experiment 10. This difference is very slight and could hardly account for the marked difference in effect on the litters. However, there is one additional outstanding difference, and that is litter size.

The litters of Experiments 8 and 10 (10 and 13 fetuses respectively) were approximately twice the size of the litter in Experiment 9. Hence, under the same conditions of exposure, it could be reasoned that there was not sufficient oxygen for the survival of the entire large litters but enough for the survival of the small litters of six.

C. Chronic Exposure.—These three experiments were designed to study the fetal mortality after exposure of the pregnant rabbit to amounts of illuminating gas relatively smaller than in the preceding experiments and over a longer period of time.

EXPERIMENT 11.—A 26-day-pregnant rabbit was exposed for a long interval each day for three

TABLE 4.—*Chronic Exposure of a Pregnant Rabbit to Illuminating Gas: Maternal Survival, Fetal Death*

Mother						Fetuses	
1st Day		2nd Day		3rd Day			
Exposure, Min.	Blood CO, %	Exposure, Min.	Blood CO, %	Exposure, Min.	Blood CO, %	Wt., Gm.	Blood CO, %
3	65	7	66	11	63	23	48
15	75	12	64	23	57	23	38
30	70	25	63	43	53	26	40
45	49	30	61	60	57	30	39
60	30	45	57	67	57	24	48
90	49	60	57	72	58	32	44
120	33						

days, being removed from the chamber long enough for a blood sample to be taken. During the periods of exposure, the rabbit became drowsy but never convulsed or became comatose. On the day following the last exposure, a litter of six was removed, all of which were dead and macerated. As seen in Table 4, the CO saturation of the fetal blood varied from 30% to 48%.

EXPERIMENT 12.—A 26-day-pregnant rabbit was exposed to illuminating gas under the same conditions as in Experiment 11; however, the total period of exposure was shorter by one hour, and each daily interval was an hour in length. The rabbit was removed briefly every 15 minutes for a blood sample. The maternal hemoglobin saturations with carbon monoxide had about the same range as those in Experiment 11, and the pregnant rabbit behaved similarly. The day after the final exposure period a litter of six was removed. Four were alive, normal, and active, with no detectable CO, and two were dead and badly macerated, showing 17% and 38% saturation of their hemoglobin with CO.

The fundamental difference between these two experiments was the duration of exposures, which was one hour longer in Experiment 11. This was on the first day, and the extent of maceration of the fetuses was consistent with death on that day. Although the litter size is the same in both experiments, exposure of one more hour caused a severe enough anoxia to kill all the fetuses in Experiment 11.

EXPERIMENT 13.—Although this experiment is of a chronic type, it differs from the above two experiments of this group in that two of the three intervals of exposure occurred on the 4th and 12th day of pregnancy and the last on the 29th. The purpose was to ascertain the effect of exposure early in pregnancy on fetal mortality. On the 29th day the pregnant rabbit was exposed to large amounts of illuminating gas, and results very similar to those in Experiment 5 were found, with the exception that the mother survived, although she convulsed and went into coma after the one-hour exposure. On the day after the last exposure a litter of six dead fetuses was removed. Five of the fetuses were slightly macerated, which was consistent with death on the 29th day, and CO saturation values of the fetuses approached the highest maternal saturation reached. One fetus was badly macerated, and its weight and development were consistent with those of a fetus of 12 days' gestation. For details see Table 5.

SUMMARY OF RESULTS

The results of the 13 experiments are summarized in Table 6.

In summary it is seen that a pregnant rabbit can be killed by exposure to large amounts of illuminating gas for a short period of time, 5 to 15 minutes, and the hemoglobin will be 82% to 84% saturated with CO, as in Experiments 1 and 2. The fetuses will survive and show hemoglobin saturations one-eighth to one-fourth of that of the maternal hemoglobin. Hence, the saturation of fetal hemoglobin lags behind that of maternal hemoglobin. Also, the pregnant rabbit may survive exposure to large amounts of illuminating gas. The hemoglobin will become 63% to 67% saturated with CO, as in Experiments 6 and 7, and she will suffer severe respiratory distress but recover. The fetuses will be normal and active, showing no effect at birth of the marked maternal anoxia.

TABLE 5.—*Chronic Exposure of a Pregnant Rabbit to Illuminating Gas on the Fourth, Twelfth, and Twenty-Ninth Gestational Days: Maternal Survival, Fetal Death*

Mother			Fetuses	
Days of Gestation	Exposure, Min.	Blood CO, %	Wt., Gm.	Blood CO, %
4	35	66	44	54
			46	49
12	18	64	53	47
			51	58
29	10	59	58	52
	30	60	10	..
	45	58		
	60	44 *		

* At this time there was a delay in obtaining the blood sample.

However, if a pregnant rabbit is exposed for a period of an hour to high concentration of illuminating gas, as in Experiment 5, with death, or in Experiment 13, with survival, all of the fetuses will be dead and the percent saturation of their hemoglobin with CO will approach the highest saturations of the maternal hemoglobin. Hence, over a long period of time the carbon monoxide saturation of fetal hemoglobin tends to reach equilibrium with that of the maternal hemoglobin, and the anoxia produced is severe enough to kill the fetuses. The maternal hemoglobin is completely cleared of carbon monoxide, whereas the fetal hemoglobin remains at a value that is slightly below the highest attained in the maternal blood.

It is also apparent that there is a marked variation in the percent saturation of the hemoglobin of dead fetuses. This is marked in the short-interval experiments, such as Experiments 3 and 4. All of the fetuses are found dead, with marked variations in the hemoglobin saturation of individuals (Table 2). In Experiments 8 and 10, 70% of the litters were born alive and the remainder dead with significant CO saturation values of their hemoglobin.

Another interesting variation is seen in Experiments 8, 9, and 10, when, under nearly the same duration of exposure, part of the litter survived in Experiments 8 and 10 and the entire litter in Experiment 9 survived. This difference can be explained by the fact that the litters of Experiments 8 and 10 were twice as large as in Experiment 9, and although oxygen was sufficient for 6 fetuses there was not enough for 10 and 13, respectively. In this respect it is of interest to note that the smaller litters of Experiments 5 and 13 showed less variation in CO-saturation values of the fetuses than in Experiments 3, 4, 8, and 10, where the size of litters were 10 or greater.

It is thought that these variations can best be explained by a difference in delivery of CO and oxygen to individual fetuses in the same uterus. Two factors must be taken into consideration: first, that a high percentage of maternal hemoglobin is incapable of carrying oxygen, since carbon monoxide is in combination with it, and hence delivery of oxygen to the fetuses will be diminished, and second, that carbon monoxide is slowly bound to fetal hemoglobin and the potential amount of oxygen it can carry is reduced. If, added to these two factors, there is a difference in distribution of the gases to the fetuses and variation in inherent individual susceptibility, the severity of anoxia of different fetuses will certainly differ. The data of the experiments were analyzed statistically for possible correlation between the weight

TABLE 6.—*Results of Exposure of Pregnant Rabbits to Illuminating Gas*

Experiment	Mother					Fetuses			
	Exposure			Blood CO *		Alive		Dead	
	Type	Min.	State	Highest Level	Mean	No.	Blood CO *	No.	Blood CO *
1	Acute	5	Dead	84	..	8	11-16	0	.....
2	Acute	15	Dead	82	..	5	17-24	1	25
3	Short intervals	59	Dead	98	71	0	.....	12	27-67
4	Short intervals	60	Dead	78	63	0	.....	10	24-58
5	Long interval	61	Dead	72	62	0	.....	6	37-65
6	Acute	13	Alive	77	53	8	0 †	0	.....
7	Acute	35	Alive	69	66	5	0 †	0	.....
8	Short intervals	12	Alive	70	66	8	0-4 †	2	40 & 40
9	Short intervals	12	Alive	66	66	6	0 †	0	.....
10	Short intervals	12	Alive	75	72	9	13-23	4	0-25
11	Chronic	252	Alive	75	56	0	.....	6	30-48
12	Chronic	180	Alive	68	51	4	0 †	2	17 & 38
13	Chronic	113	Alive	66	58	0	.....	5	47-58

* Per cent saturation of blood with CO.
† Hysterotomy done on day after exposure to gas, when living fetuses had eliminated all CO.

of the fetus and the CO saturation. There was no correlation between these factors.§

Hence, it is seen that the degree of anoxia is the major factor in explaining the results obtained by exposing a pregnant animal to illuminating gas. The time of exposure to the gas and the percentage of saturation of the maternal hemoglobin are the two principal factors responsible for the variations in the condition of the mothers and fetuses.

COMMENT

A brief review of the chemistry and physiology of CO poisoning provides an explanation of the mechanism of death for both mother and fetus in our experiments and in the cases reported in the literature.

The work of Claude Bernard in 1857 and Hoppe-Seyler in 1858, cited by von Oettingen [27] in his extensive review of CO, first proved that CO combined with hemoglobin to form carboxyhemoglobin. Douglas, Haldane, and Haldane [8] showed that the combination of CO with reduced hemoglobin and the dissociation of the carboxyhemoglobin follow the same laws that govern the formation of oxyhemoglobin and its dissociation into oxygen and reduced hemoglobin. Also, the factors affecting the formation and dissociation of oxyhemoglobin have the same effect on the formation and dissociation of carboxyhemoglobin. However, the partial pressure at which these two compounds become half saturated, their rate of formation, and their rate of dissociation vary significantly. Hemoglobin becomes 50% saturated with oxygen at a partial pressure of about 30 mm., as contrasted with 0.125 mm. for CO. Carboxyhemoglobin dissociates at a much slower rate than oxyhemoglobin. The formation of carboxyhemoglobin and oxyhemoglobin from reduced hemoglobin in the presence of both gases follows the law of mass action, and an equilibrium constant has been established. From this it is found that hemoglobin has 200 to 300 times greater affinity for CO than for oxygen. The range of 100 is due to various values reported by different workers. Killick,[17] in agreement with Anson and co-workers,[1] is of the opinion that individual variations in the affinity of hemoglobin for CO exist and are not due to experimental error. She has shown this to be true in mice, in which the affinity varied from 118 to 278. This marked affinity of hemoglobin for CO over oxygen is due to the slow dissociation of carboxyhemoglobin, although oxygen is known to combine 10 times faster with hemoglobin than CO. O'Brien and Parker [26] found that the solubility of CO in serum and plasma is of the order of 0.02 ml. per 1 ml. and agrees with its solubility in water. Hence when CO combines with and dissociates from hemoglobin, it must first dissolve in the serum. In the placenta, at the site of the villus, CO dissociates from the maternal hemoglobin, since a pressure gradient is established between maternal and fetal blood. CO then diffuses into the maternal serum, passes across the villus membrane, dissolves in the fetal serum, and combines with fetal hemoglobin. Nicloux ‖ demonstrated this by suspending a cow's caecum containing blood saturated with CO in a tank of isotonic sodium chloride in which a fish was swimming. After two hours, CO was readily detected in the fish blood. The CO had dissociated from the hemoglobin in the caecum, dissolved in the serum, crossed the membrane, dissolved in the saline, crossed the branchi of the fish, dissolved in the fish serum and combined with the fish red blood cells.

Before we consider the mechanism of fetal death by CO poisoning of the mother, the mechanism of death of the nonpregnant human by CO will be reviewed. The majority of workers agree that anoxia is the main factor in the cause of death. CO combines with hemoglobin so that oxygen no longer combines with it to form oxyhemoglobin. Hence, when the demand for oxygen by the tissues cannot be fulfilled owing to the high percentage of hemoglobin saturated with CO, the organism will die. It has been desired throughout the years to establish a

§ To be published.

‖ References 24 and 25.

definite percentage of saturation of hemoglobin with CO at which the subject will die. This has been referred to as the critical level, but it has varied considerably. Death has occurred with as low as 20% and as high as 90% saturation of the hemoglobin by CO.¶ Factors responsible for this variation are age, respiratory and circulatory diseases, a general variation from person to person of the affinity of hemoglobin for CO, and, chiefly, the length of exposure-time and the amounts of CO and oxygen in the air inspired. A definite critical level cannot be given without consideration of all the above factors. Merely the lack of reduced hemoglobin to combine with oxygen is not the only factor contributing to the anoxia. The presence of carboxyhemoglobin definitely depresses the dissociation of oxyhemoglobin, and the anoxia suffered by the tissues is definitely increased.[8] Several workers have reported that CO is a respiratory enzyme poison. Killick[17] in her review cites work indicating that death would occur because of anoxia before the partial pressure of CO in the mixture inspired would be great enough to have an effect on the respiratory enzyme.

In considering death of the fetus, anoxia is the important factor and is produced as follows: 1. Owing to saturation of the mother's hemoglobin with CO, less oxyhemoglobin will arrive in the maternal sinuses of the placenta. 2. Owing to the presence of carboxyhemoglobin, the available oxyhemoglobin in the maternal sinuses of the placenta will dissociate at a slower rate. 3. If the mother lives over a long period of time with a high saturation, the fetal blood will reach equilibrium with the maternal blood, and the amount of fetal hemoglobin available for carrying oxygen will be reduced. 4. The slow dissociation of the maternal carboxyhemoglobin as well as the fetal carboxyhemoglobin will prolong the anoxia although CO has been removed from the atmosphere. 5. Another factor offered by some workers but not fully agreed upon by all is that the fetus during initial stages of poisoning of the mother will have a higher percent of hemoglobin combined with CO than the mother, and the pressure gradient would be reversed for oxygen, and oxygen would pass from fetus to the mother. This has not been proved.[18] These factors indicate that the anoxia suffered by the fetus should be far more severe than that suffered by the mother. This is not the case in rapid gassing of the mother at high concentration of CO in the mixture inspired. Owing to the slow dissociation of carboxyhemoglobin, the fetus will have adequate oxygen, and, as we have shown, the mother, having a high level of CO in the blood, will die in a short period of time. The fetuses, having a very low level of CO in the blood, can live and exhibit little or no evidence of anoxia. Further, the work of Himwich[20] concerning anaerobic metabolism would provide an explanation for the survival of the fetuses even after oxygen disappears from the fetal blood. Conversely, when the mother is subjected to an hour or so of exposure which produces a 60% to 70% CO saturation of her blood, the fetal blood approaches and reaches that same saturation. The mother survives the exposure, but the fetuses die owing to a combination of the factors mentioned above.

¶ Stratton, F.: Personal communication to the authors.

SUMMARY AND DISCUSSION OF CLINICAL CASES REPORTED

A review of the clinical cases reported in the literature shows that they can be placed into three separate categories: 1. Both mother and fetus die. 2. The mother lives and the fetus is born dead. 3. Both survive the exposure. By applying the factors contributing to anoxia discussed above, the conditions of both mother and fetus in each case can be readily explained.

Eight cases are placed in Category 1, in which both mother and fetuses die. In five of these cases # the mother was found dead in a gas-filled room, four of these being classified as suicide. Of the remaining cases,

References 4, 6, 13, and 19.

one mother was trapped in a burning building and suffered severe burns, which no doubt contributed to her death.[19] In another case [2] the mother was present in the same room together with an adult and a child, both of whom survived. From the history, it appears that the mother was suffering from some systemic disease at the time. There is not an adequate history of the conditions pertaining to the remaining case.[6] In seven of the cases grouped in Category 1 death probably occurred in a short period of time, the mother being exposed to high concentrations of CO in the atmosphere. In all seven of the latter instances, CO levels were determined for both mother and fetus. In three of the cases * no CO was detected in the fetal blood, and the maternal blood was from 48% to 88% saturated with CO. These workers concluded that CO does not pass from maternal to fetal blood. In the remaining 4 cases † not more than one-third the level found in the maternal blood, which was sufficient to cause maternal death, was found in the fetal blood. These cases correspond to our animal Experiments 1 and 2 in which the mother died in a short period while being exposed to high concentrations of illuminating gas in the atmosphere. The maternal rabbit bloods at the time of death were 82% and 84% saturated with CO, and the fetal bloods were 11% to 24%. In summary, the death of the mother in the clinical cases was so rapid that there was not sufficient time for the dissociation of CO from carboxyhemoglobin in the maternal blood to occur; therefore, it could not be detected in the fetal blood by the methods used. Fetal death in these cases is attributed to death of the mother, with resultant fatal anoxia of the fetus after the available fetal oxyhemoglobin had been utilized and anaerobic metabolic function exhausted. It is conceivable in these human cases that if the mother had been immediately sectioned after death a living fetus could have been removed.

Six cases ‡ fall into Category 2, in which the mother survived but the fetus was born dead. The type of exposure in each one of these cases was similar and accidental, except for one, which was suicidal. In all cases the mother survived a long period of either continuous or sporadic exposure. None of the cases report a CO level of the mother's blood, but all showed classic signs and symptoms of CO poisoning as well as a history of exposure. Only two of the six cases give fetal blood levels. In one case of attempted suicide, that of Tissier,[31] one-fifth of the fetal hemoglobin was saturated with CO. The mother was found in coma in the gas-filled room and was taken to a hospital, where she underwent treatment and recovered. The anoxic condition of the mother was the cause of fetal death, and this occurred before the condition of the mother could be corrected and before an equilibrium was reached between the fetal and maternal blood. In the other case, in which the fetal blood was 49% saturated with CO, the mother showed marked signs of CO poisoning over a long period of time.§ The exposure to CO was so prolonged that fetal and maternal blood approached an equilibrium. Fetal death was due to maternal anoxia caused by a high degree of saturation of maternal hemoglobin with CO. This resulted in a diminution of oxygen to the fetus and a severe fetal anoxia, with 49% saturation of the fetal blood with CO. The amount of the fetal hemoglobin able to combine with available oxygen was reduced. The case may be compared with Experiment 11, in which the pregnant rabbit survived an hour's exposure with a high percent of CO saturation of hemoglobin. This resulted in dead fetuses with blood saturations approaching equilibrium with the maternal blood level.

The third category, in which both mother and fetus survive exposure to CO, includes 6 cases.‖ No levels are reported for the

* References 13 and 19.

† References 2 and 6.

‡ References 4, 11, 28, 30, and 31 and Muller, G. L., and Graham, S.: To be published.

§ Muller, G. L., and Graham, S.: To be published.

‖ References 3, 4, 7, 18, 20, and 34.

adults or fetuses. However, the clinical signs, symptoms, and histories of the mothers are those of classic CO poisoning, and in three cases it was definitely established that the women were unconscious for five hours or longer.¶ In one report,[4] two pregnant women occupied the same room in a maternity hospital and were exposed to the gas overnight. One of the women, three to four weeks from term, delivered a stillborn fetus on the following day. The other delivered a perfectly normal healthy baby one month after exposure to the gas. The latter was closer to the window and in a deep sleep during the gassing. It is postulated that she received more oxygen than the other, who was violently ill. In two of the remaining cases the baby died a short time after birth. Maresch [15] reported that a young woman attempted suicide and was found unconscious in a gas-filled room. She was revived at a hospital and delivered a living baby 13 days after the poisoning. The child died nine days later and at autopsy had softening in the basal ganglia. This is the classic pathological lesion found in the human who survives severe CO poisoning after being comatose for hours to days, then subsequently recovers, and survives for months to years with symptoms related to lesions of the basal ganglia. In the case of Neuburger,[20] the mother suffered from acute CO poisoning and was unconscious for five hours. The child was born five weeks after the poisoning and lived only six days. Its brain was smaller than the inner skull, and there was considerable hydrocephalus internus. The author reports the brain abnormalities as due to CO intoxication. In the case documented by Brander,[3] in which the pregnant woman was exposed to the gas in the fourth month and was unconscious for five hours, a live baby was delivered at term. However, from birth the child showed definite neurological signs and symptoms, which persisted to the age of four, when the report was written. The child was diagnosed as microcephalus (idiota) and tetraplegia (Littlesches syndrome), which Brander claims in this case is due to CO poisoning and not heredity. Desclaux and co-workers [7] report a similar case of CO poisoning in the fifth month of pregnancy, and it is stated that the mother was in coma and rescued before prolonged exposure had occurred. At the time of writing the child was 8 years old and classified as an idiot. Zourbas [24] cites a case of accidental poisoning of a woman at the end of three months of pregnancy. She was comatose for more than nine hours. The child was born alive at term, lived for eight months, and exhibited a retardation of psychomotor development. No autopsy was done in this case, and brain damage was thought to be due to CO by Zourbas, who felt that the neurological signs and symptoms could be placed in the same regions of the brain affected by classic CO poisoning. In these six cases, the survival of the fetus is probably due to the fact that neither was the anoxia of the mother too severe nor had the fetal blood reached equilibrium with the maternal blood. These cases could be compared to our Experiments 8, 9, and 10, in which the pregnant rabbit was exposed to high concentrations of illuminating gas for short periods of time under about the same conditions. In Experiments 8 and 10 a small part of the litter was born dead, and in Experiment 9 all were born alive. We feel that in Experiments 8 and 10 the anoxia was not severe enough to kill all the fetuses but that there was not adequate oxygen in order for all to survive. There may be considered a borderline condition in maternal CO poisoning in which the fetus is just able to survive, and if the anoxia be slightly more severe the fetus will die.

In summary, it may be stated that the condition of the fetus and mother in CO poisoning can be explained by the severity of anoxia, which is directly related to the amount of CO in the mixture inspired and the duration of exposure. Theoretically, a baby could be removed alive if the mother is discovered immediately after death when exposed for a short period to high concentrations of CO. Also, if a mother is exposed

¶ References 3, 4, and 20.

to CO in the last three weeks of gestation and shows definite signs and symptoms of poisoning, it is possible that a live premature baby might be delivered by immediate Caesarean section.

CONCLUSIONS

Carbon monoxide passes from maternal to fetal blood.

The percentage of saturation of fetal hemoglobin with CO is dependent upon the percentage of saturation of the mother's hemoglobin with CO *and the length of time of exposure to the gas.*

The saturation of fetal hemoglobin with CO lags significantly behind the saturation of the maternal hemoglobin, owing to the slow dissociation of maternal carboxyhemoglobin. After a period of time an equilibrium is approached between fetal and maternal blood.

Animal experiments are presented in which exposure time of the mother to CO and the concentration of CO in the mixture were varied. In this manner all the conditions previously reported in animal experimentation and in clinical cases have been simulated.

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News and Comment

ANNOUNCEMENTS

Postgraduate Assembly in Endocrinology and Metabolism.—The Seventh Postgraduate Assembly in Endocrinology and Metabolism sponsored jointly by the Endocrine Society and the Indiana University School of Medicine will be held at the Indiana University Medical Center, Indianapolis, Sept. 26 to Oct. 1.

The faculty will consist of 21 eminent clinicians and investigators from various parts of the country in the fields of endocrinology and metabolism. The program will cover the various endocrinopathies, with emphasis on the clinical aspects, demonstration of laboratory tests, presentations of cases, and question-and-answer panel discussions. The course is designed to cover the main aspects of diagnosis and therapy in the field of endocrinology and metabolism for the physician in general practice and for those in other specialties who wish to have a general knowledge of this rapidly growing field.

A syllabus with brief abstracts of lectures will be available to the registrants at the time of the assembly.

For further information concerning the program, write Postgraduate Office, Indiana University Medical Center, 1100 W. Michigan St., Indianapolis 7. Registration is limited to 100; tuition fee is $100. Arrangements for housing accommodations will be made through the University.

Steroid Hormone Effects on Adrenal Dehydrogenase Activity

In Vitro Experiments with Control and Tumor-Bearing Mice

MAURICE M. BLACK, M.D.
and
FRANCIS D. SPEER, M.D., New York
With the Technical Assistance of Jean Watts, B.A.

In a recent paper we described the effects of glucocorticoids and mineralocorticoids on the in vitro dehydrogenase activity of adrenal gland slices from control and tumor-bearing CFW mice.[1] The dehydrogenase activity of the zona glomerulosa of adrenal slices from control animals was characterized by a tendency to be inhibited by desoxycorticosterone acetate (DOCA) and stimulated by cortisone and Compound F (17-hydroxycorticosterone). In contrast, the dehydrogenase activity of the zona glomerulosa of adrenal slices from mice bearing spontaneous mammary carcinomas tended to be inhibited by glucocorticoids and unaffected or stimulated by DOCA. To some extent the findings in the outermost zona fasciculata tended to parallel those observed in the zona glomerulosa. No appreciable changes were noted in the inner zona fasciculata or zona reticularis.

In view of the well-defined in vitro effects of these steroid hormones, it seemed desirable to extend such observations to include other steroids having different physiological properties. The present communication is a report of the effects of testosterone, estradiol and Compound A (11-dehydrocorticosterone) on the in vitro dehydrogenase activity of adrenals from control and tumor-bearing CFW mice. The data provide further evidence of differences in the reactivity of the various cortical zones, sex differences in the metabolism of the control adrenals, and further evidence of adrenal changes associated with the presence of spontaneous mammary tumors.

From the Department of Pathology, New York Medical College, Flower and Fifth Avenue Hospitals.

The investigation was supported by the Helen Andreadis Foundation, Inc., and the Leukemia Research Foundation, Inc.

MATERIALS AND METHODS

Three series of CFW mice were employed in the present study. They consisted of 13 control nongravid females weighing 20.8 to 36.2 gm. (mean weight, 28.8 gm.), 16 control males weighing 26.2 to 38.9 gm. (mean weight, 33.8 gm.), and 25 nongravid females bearing spontaneous mammary tumors. The tumor mice weighed between 22.5 and 40.3 gm. (mean weight 32.2 gm.), while the tumor weights varied from 0.3 to 3.5 gm. (mean weight, 1.0 gm.).

The animals were killed by rapidly crushing the cervical spine. The adrenals were then removed, gently cleared of their surrounding fat, and bisected with a razor, and one-half of an adrenal was placed in the appropriate incubation solution.

The incubation solution employed for the determination of the endogenous in vitro dehydrogenase activity (R_e) consisted of 3 ml. of a 1% aqueous solution of 2,3,5-triphenyltetrazolium chloride (TTC) buffered to pH 7.2 and 1 ml. of 1.9% sodium chloride. In the studies with the steroid hormones, 1 ml. of a supersaturated aqueous solution of crystalline hormone * was substituted for the isotonic saline. The final mixtures thus obtained yielded a fine dispersion containing approximately 0.2 mg. of estradiol (R_e), 5 mg. of testosterone propionate (R_t), and 0.1 mg. of Compound A (R_a) in the respective incubation media.

* The crystalline estradiol and testosterone propionate were supplied by the Syntex Division of Chas. Pfizer & Company, Inc.

After incubation for one hour at 37 C, the reactions were stopped by fixing the tissues in 10% formalin. After fixation, frozen sections were prepared. In almost all cases four sections from each adrenal slice were examined. Color photomicrographs were made of each determination.

An evaluation was made of the intensity of staining, as seen in the sections of the adrenocortical zones under the different experimental conditions. In addition, after the series had been completed, the color photomicrographs were used to reevaluate the relative intensity of dye reduction under the various experimental conditions. The intensity of staining in the different zones was graded from 0 to 4+. A bright red color was assigned a grading of 4+; the absence of color was graded a 0; while intermediate intensities of color were assigned values

Relative Intensity of Dehydrogenase Activity of Adrenocortical Zones in Presence and Absence of Steroid Hormones

Zone and Group	Measurement			
	R_o	R_e	R_t	R_a
Glomerulosa				
Male, control	1.94	1.06	1.87	0.81
Female, tumor	2.52	1.58	1.47	1.25
Female, control	1.00	1.07	1.08	0.54
Fasciculata				
Male, control	5.24	2.50	3.18	2.62
Female, tumor	3.52	2.84	2.66	3.00
Female, control	3.07	2.71	2.67	2.27
Reticularis				
Male, control	3.00	2.44	2.94	2.62
Female, tumor	3.46	3.22	3.24	3.20
Female, control	2.46	2.78	2.84	2.54

in accord with their relation to the two extremes of the scale. It should be mentioned that replicate evaluations of the same slides yielded very similar values. Deviations averaged less than ±½.

RESULTS

In the accompanying Table we have indicated the mean values obtained in the cortical zones of the adrenals in the presence and absence of the various hormones studied. It is evident that a differential sensitivity exists in the various cortical zones. Thus, the zona glomerulosa exhibited the maximal sensitivity to the steroid hormones employed, whereas the zona reticularis showed no appreciable change. The zona fasciculata tended to be altered in the same direction as the glomerulosa zone, but to a less degree. It should also be mentioned that there was a graded response within the fasciculata zone. The outermost portion responded more similarly to the glomerulosa zone, while the innermost portion was more similar to the reactions of the reticularis zone.

Male Controls.—As indicated in previous reports, the three cortical zones exhibit a high degree of endogenous dehydrogenase activity.† In the adrenal slices incubated in the presence of testosterone, there was no appreciable change as compared to the endogenous activity. In contrast, the slices incubated in the presence of Compound A revealed a marked inhibition of the staining of the zona glomerulosa, slight inhibition of the zona fasciculata, and only questionable inhibition of the zona reticularis. Adrenal slices incubated with TTC plus estradiol revealed a marked inhibition of the zona glomerulosa, slight effect on the zona fasciculata, and minimal effect on the zona reticularis.

Female Controls.—As indicated previously, the adrenals of the control female CFW mice are characterized by a rather low intensity of dehydrogenase activity in the zona glomerulosa, a prominent activity in the fasciculata zone, and a variable activity in the reticularis zone.‡ The latter zone is weakly active in younger mice but becomes more active in older animals.[1]

The effect of testosterone on the adrenal slices from the female control mice was similar to that found on the male adrenal slice, viz., minimal effect. As with the male adrenals, Compound A caused a well-defined inhibition of the glomerulosa, slight inhibition of the fasciculata zone, and no effect on the zona reticularis. However, in contrast to the findings in the male group, estradiol did not produce any significant decrease in intensity of staining in any of the zones of the adrenals from the female controls.

Female Mice with Spontaneous Mammary Tumors.—The adrenal slices from female CFW mice bearing spontaneous mammary tumors are characterized by a high degree of in vitro endogenous reduction of TTC.§

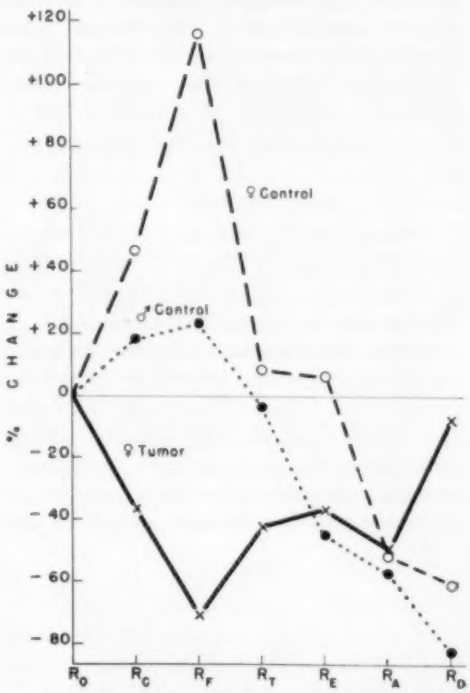
† References 1 and 2.
‡ References 1 and 2.
§ References 1 and 2.

However, as will be seen in the accompanying Table, testosterone, estradiol, and Compound A all produced well-defined inhibition in the zona glomerulosa. A slight degree of inhibition was also observed in the fasciculata zone, whereas no change was found in the zona reticularis.

COMMENT

It would appear from the above data that the zona glomerulosa is much more sensitive to the hormones studied than the other cortical zones. These observations parallel our previous findings in regard to the effects of cortisone, Compound F, and DOCA.[1] However, it should be noted that the zona fasciculata tended to show a gradation in response with the outermost region, tending to behave in a similar fashion to the glomerulosa zone, while the innermost zone was more similar to the zona reticularis. In the reticularis zone neither estradiol nor testosterone caused any demonstrable effect on the dehydrogenase activity in either the male or female controls or in the tumor-bearing mice. It is recognized, however, that changes may have occurred which were not visualized by the present method of study.

In our initial study of the endogenous dehydrogenase activity of the adrenals of control and tumor-bearing mice we commented that the distribution of staining in the tumor-mice adrenals was more similar to that of the male group.[2] The current observation that estradiol inhibited the TTC reduction in the adrenal slices of the control males and tumor-bearing females, but not those of the control females, would be in line with the above observations. However, the situation appears to be complex, since testosterone inhibited the tumor-mice adrenals but did not affect the control males or females. In the accompanying Figure we have presented a summary diagram of the effects of the different steroid hormones studied thus far on the zona glomerulosa of male and female controls and female tumor-bearing mice. It is evident that the adrenals of the three groups reveal distinctive metabolic profiles which show the following salient features: (*a*) glucocorticoid stimulation and mineralocorticoid inhibition of the control male and female adrenals, i. e., a higher TTC reduction in the presence of glucocorticoids than in the presence of mineralocorticoids; (*b*) a reverse tendency in the adrenals of the tumor-bearing females; (*c*) estradiol inhibition of TTC reduction by the adrenals of male and tumor-bearing female mice, but no such effect on female control adrenals; (*d*) testosterone inhibition of TTC reduction



Influence of steroid hormones on in vitro dehydrogenase activity of zona glomerulosa in control and tumor-bearing mice. Per cent changes from endogenous values.

by adrenals of tumor-bearing mice but no effect on control males or females; (*e*) a similar degree of Compound A inhibition of TTC reduction by adrenals of all three groups.

Some comment should also be made in regard to the degree of uniformity of response encountered in the individual cases studied. In all the determinations the majority of the individual adrenals in the various groups behaved in a fashion which

paralleled the effect indicated by the mean changes for the group. However, some variation in the degree of such change was encountered, and in an occasional instance a contrary type of response was encountered. It would be of interest to know to what extent individual variation in the adrenal metabolism of the tumor mice is related to the behavior of the tumors in such cases. Work along this line is in progress.

Finally, these data provide additional evidence of the complex changes in adrenocortical metabolism in the tumor host. Further, they demonstrate the ability of the tetrazolium techniques to visualize subtle metabolic differences in functional states. Further work along this line appears indicated.

SUMMARY

A histiochemical study was made of the in vitro dehydrogenase activity of adrenal slices of CFW mice, utilizing the tetrazolium salt 2,3,5-triphenyltetrazolium chloride (TTC). A comparison was made between the intensities of the metabolic activity of the various cortical zones as found in adrenals from male and female controls and females bearing spontaneous mammary cancer. In addition, an evaluation was made of the relative effects of testosterone propionate, estradiol, and Compound A on the dehydrogenase activity in the above groups.

The dehydrogenase activity of the zona glomerulosa of adrenal slices from the control and tumor-bearing animals was characterized by a tendency to be inhibited by Compound A. Estradiol produced no appreciable change on the dehydrogenase activity of adrenal slices from female control mice, whereas definite inhibition was observed in adrenal slices from male control and tumor-bearing female mice. Testosterone failed to change the intensity of staining in adrenal slices from control males or females but did cause inhibition of the slices from the tumor-bearing females. These findings and our previous studies provide evidence of differences in adrenal cortical metabolism in relation to sex and the presence of the spontaneous tumor studied. The findings in the outermost zona fasciculata tended to parallel those observed in the zona glomerulosa. No appreciable changes were noted in the inner zona fasciculata or zona reticularis.

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A Delayed Allergic Reaction in the Knee Joint and Hindfoot of the Guinea Pig

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and
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The production of a delayed allergic inflammatory reaction in the guinea pig hindfoot has been reported.[1] In the present study a similar response was elicited in the knee joint of the guinea pig, with use of a comparable technique, namely, two simultaneous preparatory injections, one of antigen alone into the joint space (second depot) and the other of the same antigen incorporated in Freund's adjuvants for systemic sensitization. In addition, data are included on the delayed allergic response in the hindfoot, with special reference to dosage as well as the detection of residual antigen in the foot depot when followed one or two weeks later by a corresponding sensitizing emulsion.

MATERIALS AND METHODS

Light-skinned male guinea pigs of mixed breed, weighing 600 to 800 gm., were used. Systemic sensitizing emulsions prepared in a Waring Blendor consisted of rabbit serum or crystallized hen's ovalbumin incorporated in Bayol-F (Standard Oil Co. of New Jersey) containing either Arlacel A (Atlas Powder Co.) or Falba (Pfaltz & Bauer) in which dried autoclaved Mycobacterium tuberculosis (Jamaica 22*) had been suspended. Injections were made either subcutaneously in the nuchal region (three sites totaling 2 ml.), or infracutaneously on the upper ventral thorax, as more recently described by Lipton and Freund,[2] using two sites of 0.1 ml. each.

For eliciting the reaction at the knee joint, 0.3 ml. of rabbit serum was injected through the intact clipped skin into the synovial cavity, with the animal under pentobarbital (Nembutal) anesthesia, within several hours of administration of sensitizing emulsion.

For preparation of the hindfoot, antigen (crystallized ovalbumin or rabbit serum) was injected in a volume of 0.2 ml. each on three successive days. The corresponding sensitizing emulsion was administered on the 1st, the 8th, or the 15th day.

Clinical responses at the knee joint or foot depot were graded ± to 4+, depending on the degree of inflammation observed externally (erythema, swelling), enlargement of the joint, and either partial or complete disuse of the affected joint or foot. Caliper measurements proved useful for estimating the amount of swelling at the knee joint. Initially, a nonspecific reaction was observed in the injected foot for several days following preparation, but this always subsided before the onset of the specific allergic response. Scores as recorded in the accompanying Tables were obtained by plotting on ordinary graph paper readings taken on at least four days per week and measuring the area of response.

RESULTS

A delayed inflammatory reaction was elicited in the knee joint of a majority of 33 guinea pigs in which rabbit serum had been injected into the synovial cavity and a rabbit serum-adjuvant emulsion containing killed M. tuberculosis administered either subcutaneously or intracutaneously within several hours (Table 1). When killed M. tuberculosis was omitted from the adjuvant emulsion, only one of five guinea pigs reacted. No response was observed in control animals in which crystallized ovalbumin was substituted for rabbit serum in the adjuvant emulsion administered either simultaneously or four to six months prior to the injection of rabbit serum into the knee joint.

The average onset of the knee joint reaction was somewhat earlier when the intra-

From the Department of Bacteriology, New York State Psychiatric Institute.

* Furnished by Dr. Jules Freund.

TABLE 1.—*Delayed Allergic Response in Knee Joint of Guinea Pig*

	Adjuvant Emulsion					Second Depot					
Exp. No.	Rabbit Serum, Ml.	Ariacel A, Ml.	Bayol F, Ml.	TB, Mg.	Route	Rabbit Serum, Ml.	Depot Route	No. POS/ No. Tested	Avg. Onset, Days‡	Avg. Score POS	Avg. Score TOT
1	1.0	0.15	0.85	1.0	Sub. nuchal	0.3	Knee	5/14	21	10	4
1	0.1	0.015	0.085	0.2	I. C. thorax	0.3	Knee	11/15	13	24	18
2	0.1	0.015	0.085	0.2	I. C. thorax	0.3	Knee	3/4*	14	..	..
3	0.1	0.015	0.085	0.2	I. C. thorax	0.3	Knee	6/6*	14	..	..
2	0.1	0.015	0.085	0	I. C. thorax	0.3	Knee	1/5*	15	..	..
	Ea 1 Mg.	Controls									
2	0.1	0.015	0.085	0.2	I. C. thorax	0.3	Knee	0/4	..	..	..
3†	0.1	0.015	0.085	0.2	I. C. thorax	0.4	Knee	0/3	..	..	..

Sub. indicates subcutaneous; I. C., intracutaneous; Ea, crystallized hen's ovalbumin; POS, positive reactors; TOT, total group.

* Killed at various intervals after onset of reaction for histologic examination.

† Adjuvant emulsion injected 4 to 6 months prior to knee joint injection.

‡ Observed 27 to 30 days after onset.

Remaining negative reactors under observation 1½ to 5 months.

TABLE 2.—*Delayed Allergic Reaction in Hindfoot of Guinea Pig Produced with Crystalline Hen's Ovalbumin—Dosage*

Adjuvant Emulsion* Ea, Mg./0.1 Ml.	Foot Depot Ea, Mg./0.6 Ml.†	No. POS/ No. Tested	Avg. Onset, Days	Avg. Score
13.0	77	5/5	8	80
1.3	77	3/3	7	80
0.13	77	1/1	18	37
0.013	77	2/3	17	18
1.3	7.7	4/4	15	78

Ea indicates hen's ovalbumin crystallized 4× from sodium sulfate (mg. N×6.25).

* 0.2 ml. injected intracutaneously at two sites (0.1 ml. each) on upper ventral thorax on first day; total dose contained 0.1 ml. Ea, 0.015 ml. Ariacel A, 0.085 Bayol F, 0.1 mg. killed M. tuberculosis.

† 0.6 ml. = 3 injections of 0.2 ml. on first 3 days.

cutaneous route was employed for systemic sensitization as compared with the subcutaneous route. The duration of the observed inflammatory response varied from 3 days to 1 month or more with intracutaneous sensitization; while in those sensitized subcutaneously, three animals showed a response for 1 day only, one for 7 days, and one for 14 days. A comparison between the two routes is not intended here, since the dosages employed in the adjuvant emulsions were not the same.

A similar delayed inflammatory reaction was elicited in the hindfoot of the guinea pig for the purpose of determining optimal dosages, with use of crystallized ovalbumin as antigen. Table 2 shows that for incorporation in the adjuvant emulsion injected intracutaneously, doses of 13 and 1.3 mg. were highly effective when 77 or 7.7 mg. of ovalbumin was employed as the second antigen depot. However, in the case where the smaller amount was employed in the foot depot, the onset of the reaction was delayed, averaging 15 days as compared with 7 to 8 days. Emulsions containing 0.13 and 0.013 mg. of ovalbumin in the presence of 77 mg. of ovalbumin in the second depot yielded lower degrees of reactivity with delayed onset. Although the titration is incomplete, the data are presented as preliminary observations.

TABLE 3.—*Detection of Antigen Depot in Hindfoot of Guinea Pig by Subsequent Systemic Sensitization*

	Second Depot Hindfoot, Rabbit Serum			Adjuvant Emulsion* Nuchal Region, Rabbit Serum						
Day 1 Ml.	2 Ml.	3 Ml.	1 Ml.	8 Ml.	15 Ml.	No. POS/ No. Tested	Onset, Days†	Maximum Intensity	Avg. Score
0.2	0.2	0.2	0.8	...	...	4/4	12-19	3+-4+	49
0.2	0.2	0.2	...	0.8	...	4/4	12-19	4+	70
0.2	0.2	0.2	...	...	0.8	3/3	19-26	2+-4+	34

* 2 ml. injected subcutaneously, containing 0.8 ml. rabbit serum, 0.4 ml. Falba, 0.8 ml. Bayol F, 1.6 mg. TB.

† After adjuvant emulsion.

The following attempt was made to estimate the amount of residual reactive antigen in the foot depot after one or two weeks (Table 3). Rabbit serum was injected on the first 3 days into one hindfoot of 11 guinea pigs, while a rabbit serum-adjuvant emulsion was administered subcutaneously in the nuchal region on the 1st, 8th, or 15th day. A delayed inflammatory reaction in the prepared foot occurred in every instance. Although the data are limited because of the small number of guinea pigs available, it would appear that a significant amount of residual antigen remained in the foot depot for a considerable number of days. It is believed that the greater response of the second group of animals may be attributed to the spacing of injections in which the adjuvant emulsion served as a booster dose. This is not evident in Group 3, where residual antigen in the foot may have diminished below the optimum for maximal reactivity.

SUMMARY

A delayed allergic inflammatory reaction was produced in the knee joint of the guinea pig after a single injection of rabbit serum into the synovial cavity, when accompanied by simultaneous systemic sensitization with rabbit serum in Freund's adjuvants. Preliminary data are included to show the effect of varying the concentration of antigen either in the sensitizing emulsion or in the second depot (hindfoot), employing crystallized hen's ovalbumin as antigen. A delayed allergic inflammatory reaction was elicited in the hindfoot in which rabbit serum had been injected one or two weeks prior to the subcutaneous nuchal injection of a rabbit serum-adjuvant emulsion.

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News and Comment

ANNOUNCEMENTS

Tenth Rheumatism Review.—The Tenth Rheumatism Review, a comprehensive review of the American and English literature of recent years, is being made available for the first time to the medical profession at cost by the Arthritis and Rheumatism Foundation.

Physicians may obtain their copies of the Review by sending $1.00 to the Arthritis and Rheumatism Foundation, 23 West 45th St., New York 36.

The 418-page Review is based on material culled from 2,250 medical and scientific papers published in the field of arthritis and the rheumatic diseases over a five-year period. Written in straight prose and marked for reference, it was prepared by the Editorial Committee of the American Rheumatism Association, the professional society in the field. The book comes complete with index and bibliography.

American Association of Pathologists and Bacteriologists.—At the recent meeting of the American Association of Pathologists and Bacteriologists held in Houston, Texas, April 7, 8, and 9, 1955, the following officers were elected: President, Dr. Edwin W. Schultz; Vice President, Dr. Granville A. Bennett; Secretary, Dr. Edward A. Gall; Treasurer, Brigadier General Elbert DeCoursey; Incoming Member of Council, Dr. Douglas H. Sprunt.

The next scheduled meeting of the Association will be held in Cincinnati, Ohio, April 26, 27, and 28, 1956. The symposium, which will be given at this meeting is to be entitled, "Virus Diseases." The moderator will be Dr. Albert B. Sabin.

Neoplasms of the Subcoelomic Mesenchyme

Report of Two Cases

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and
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The subcoelomic mesenchyme may be defined as the layer of tissue which lies underneath the mesothelial surface of the peritoneum. In the primitive pelvic coelom this layer of tissue gives rise to the mesenchyme of the urogenital ridge which surrounds the Müllerian and Wolffian ducts. The epithelium which lines these ducts is formed by invaginations of the coelomic lining, and further contributions to it are made by the stromal cells. In subsequent organogenesis the development of both the urinary and the genital tracts obscures the persistence of the purely mesenchymal component in the adult except for the endometrial stroma to which it is closely related.

In the adult the subcoelomic mesenchyme is represented by an inconspicuous layer of flattened cells which merges imperceptibly into the subserosal stroma of the uterus, ovaries, tubes, and the uterine ligaments. It is somewhat more prominent over the dome of the bladder and on the pelvic parietes. On occasion the presence of the mesenchymal cells may be made visible by their participation in some tissue reaction, of which the most obvious example is the formation of ectopic decidual plaques in pregnancy. These have been observed in such diverse locations as the appendix, the omentum, the renal pelvis, beneath the subcapsular sinus of pelvic lymph nodes, as well as the more usual locations underneath the peritoneal surfaces of the ovaries, Fallopian tubes, and uterus. Ectopic decidual reactions have been described in the absence of pregnancy; their occurrence merely indicates that the ability of cells of the subcoelomic mesenchyme which have been sensitized by estrogenic substances and subsequently stimulated by progesterone or other progestational substances to form decidual cells is a general property of these cells and is not specific for the pregnant state. In this respect their kinship to endometrial stroma (the least specialized tissue of the adult female genital tract) is clearly shown. Likewise, the subcoelomic mesenchyme participates in endometriosis; Weller [1] was among the first to point out that the distribution of endometriosis in the female pelvis corresponds to the distribution of ectopic decidual reactions. The so-called endometriosis of pelvic lymph nodes probably represents inclusions of coelomic lining cells and their surrounding mesenchyme in the formation of these lymph nodes.

Even in less complex lesions can the presence of the subcoelomic mesenchyme be detected. The formation of peritoneal adhesions in general is a function of this tissue, and again in the pelvic peritoneum this can be most clearly seen when a decidual reaction occurs in such adhesions, thereby furnishing a clue to the genealogy of the cells involved. The occurrence of such decidual reactions in pregnancy has been described by many observers, and in the absence of pregnancy by Ober, Grady, and Schoenbucher.[2]

However, the role of the subcoelomic mesenchyme in tumor formation seems less well defined. Ober and Jason [3] reported a case of sarcoma of the endometrial stroma in a 43-year-old woman who had a large polypoid sarcoma in the endometrial cavity and sarcomatous transformation of the stromal component of endometriosis of the serosal

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surface of the Fallopian tube. This was interpreted as concurrent multicentric origin of the sarcoma. Neoplastic transformation of endometriosis is quite uncommon. Corner, Hu, and Hertig [4] found only 8 previously reported cases of carcinoma arising in endometriosis, and in 265 cases of ovarian malignant neoplasm they found only 3 additional cases of carcinoma arising in endometriosis. They were not able to find any case of sarcomatous transformation of the stromal component. Subsequently, MacFarlane and Pritchard [5] reported a case of Müllerian carcinosarcoma arising in an endometrial cyst of the ovary, comparing its histologic features with a similar carcinosarcoma of the endometrium.

Although sarcomas of the female genital tract are infrequent, they were well recognized and clearly defined almost a century ago. Zenker [6] in 1864 classified these tumors into "pure" and "mixed" varieties and stated that they might contain only homologous tissue elements or might contain heterologous elements. This classification is still valid. The "pure homologous" type is the endometrial stromal sarcoma. A variant of this is endolymphatic stromal myosis (stromatous endometriosis), in which the capacity of the "juvescent" stromal cells for autonomous growth and metastasis is somewhat limited. The "pure heterologous" type include such tumors as rhabdomyosarcoma or chondrosarcoma of the uterus, cervix, or vagina, in which the greatest portion of the tumor is composed of cells differentiating as muscle or cartilage. The "mixed" tumors include such entities as mixed mesodermal tumor (the combined mesenchymal sarcoma and carcinoma of Hill and Miller [7] and the malignant mixed Müllerian tumor of Sternberg, Clark, and Smith [8]), which is usually found in women over 40, and its juvenile variety, sarcoma botryoides which tends to rise in the upper vagina of prepubescent girls and the cervix of young women. Mixed tumors may be composed of purely homologous elements, e. g., a carcinosarcoma of the endometrium which contains only adenocarcinoma and sarcoma of the endometrial stromal type, or they may contain a wide variety of heterologous elements (neoplastic smooth muscle, striated muscle, cartilage, bone, etc.). The latter vary in both their quantity and distribution in the primary tumor as well as in metastatic deposits within the range of cellular transformation of mesenchyme.

Sarcoma arising in the pelvic supporting tissues outside the uterus, cervix, and vagina with no tumor present in these organs is quite rare. The case reported by Ober and Jason [9] and the two cases reported below are the only examples of this sort of tumor which we have been able to find.

CASE REPORTS

Case 1.—N. S. (St. Luke's Hospital S-53-2018, S-53-2113, A-53-103), a 74-year-old white woman, was first admitted on June 12, 1953, because of diffuse pelvic pain, frequency and dysuria, and pain on defecation. These symptoms had been present for six months but had become severer during the previous two months. There was no weight loss or vaginal bleeding. Menopause had occurred uneventfully 24 years previously. She had never been pregnant. A uterine suspension and left salpingo-oöphorectomy had been performed in 1912. An appendectomy and resuspension of the uterus had been performed in 1917. Physical examination was unremarkable except for moderate obesity, abdominal distention, and suprapubic tenderness, as well as moderate tenderness over the umbilicus and epigastrium. The vaginal mucosa was atrophic and a 5-cm. soft bluish tumor bulged into the vault; it was not ulcerated and appeared to be covered by intact epithelium. The cervix was not visualized and its relation to the mass could not be made out. The uterus was slightly enlarged and was separate from the tumor felt through the vaginal vault. No masses were palpated in either adnexal area. Additional studies were not remarkable. A portion of the tumor was excised *per vaginam* for microscopic examination on June 16, and a report of undifferentiated malignant neoplasm was made. On June 22 a laparotomy was performed. The pelvis was filled with a mass which appeared to "replace the uterus." The right ovary and tube were identified and were separate from the neoplasm. The left tube and ovary were absent. The mass and the right adnexae were excised; it was felt that residual tumor was present after the operation.

Following postoperative radiation therapy, the patient was discharged after an uneventful convalescence. She remained in good health for three months, but in November, 1953, abdominal disten-

tion reappeared and a fist-sized mass was felt in the right lower quadrant. Ascites developed, and two paracenteses removed a total of 7500 cc. of serosanguinous fluid. She became progressively weaker and died on Nov. 21, five months postoperatively. Postmortem examination was performed.

PATHOLOGIC OBSERVATIONS

The first specimen (S-53-2018) consisted of about 25 gm. of large and small fragments of soft, friable, homogeneous, pale tan tissue mottled with bright red areas of hemorrhage. Microscopic examination revealed sheets of large cells arranged in no particular pattern. The nuclei were round, oval, or reniform, with finely divided chromatin. The cytoplasm was inconspicuous. Many mitotic figures were seen. The scanty stroma consisted of a few small bands of connective tissue and some small blood vessels. Because of occasional suggestive palisading of the neoplastic cells about the thin bands of stroma and because of the greater frequency of carcinoma than sarcoma, a diagnosis of undifferentiated malignant neoplasm, probably carcinoma, was made.

The specimen removed at laparotomy (S-53-2113) consisted of a 100 gm. uterus, including the cervix with the right tube and ovary attached. Most of the right side of the uterus was encased by soft grayish-tan, ragged, focally hemorrhagic tumor tissue. The tube and ovary were separate from the tumor which encircled the uterus and cervix and extended downward along the right paracervical area to the plane of excision. The tumor tissue did not penetrate the uterine or cervical wall to any greater depth than 0.2 cm. The endocervical canal and endometrial cavity were patent and lined by thin, glistening mucous surfaces. Submitted separately were many irregular pieces of tumor tissue with an aggregate weight of 30 gm.

Microscopic examination of this specimen revealed sheets of undifferentiated malignant tumor similar to that seen in the previous surgical specimen. These surrounded the uterus and cervix and were seen extending into the outermost layers of the myometrium along the connective tissue septa between muscle bundles. Clumps of tumor cells were also seen lying within occasional lymphatic vessels at the periphery of the myometrium. The mucous membranes of the endometrium, endocervix, and exocervix were unremarkable except for senile atrophy and a small sessile endometrial polyp showing hyalinized stroma and occasional cystically dilated glands. In the parametrium, the connective tissue was extensively displaced and disrupted by large sheets of undifferentiated malignant cells growing without pattern. Admixed with these were mature differentiated tubular structures, lined by a single layer of columnar epithelial cells surrounded by thin coats of collagenous connective tissue. In one area these tubules were markedly numerous and gave the impression of a pseudoadenomatous formation. There was no hyperplasia, piling up, or papillary infolding of the epithelium, merely an apparent increase in quantity. Because of their location and appearance these tubular structures were identified as vestigial remnant of the Wolffian apparatus. At the peritoneal surface, a papillary pattern was evident. The stroma of the papillae was composed of compressed tumor cells which assumed a spindly appearance, and the surface of the papillae was composed of cuboidal cells which occasionally assumed a single-layered pattern of complex infolding and lacuna formation, but which also showed evidence of proliferation into small sheets and pseudoacini. The cuboidal cells covering the papillae merged imperceptibly into the flat layer of peritoneal cells. In nearby areas of the peritoneal surfaces there were metaplasia of the mesothelium and a surrounding transformation of the subjacent stroma which suggested an atypical pattern of endometriosis.

The autopsy (A-53-103) revealed pertinent findings restricted to the abdominal cavity, which contained 2000 cc. of serosanguineous fluid. Almost the entire visceral peritoneum was thickened by confluent masses of grayish-white, friable tumor which encased the large and small intestines and produced numerous dense adhesions of the omentum to the abdominal wall and bowel, as well as adhesions of loops of bowel to other loops of

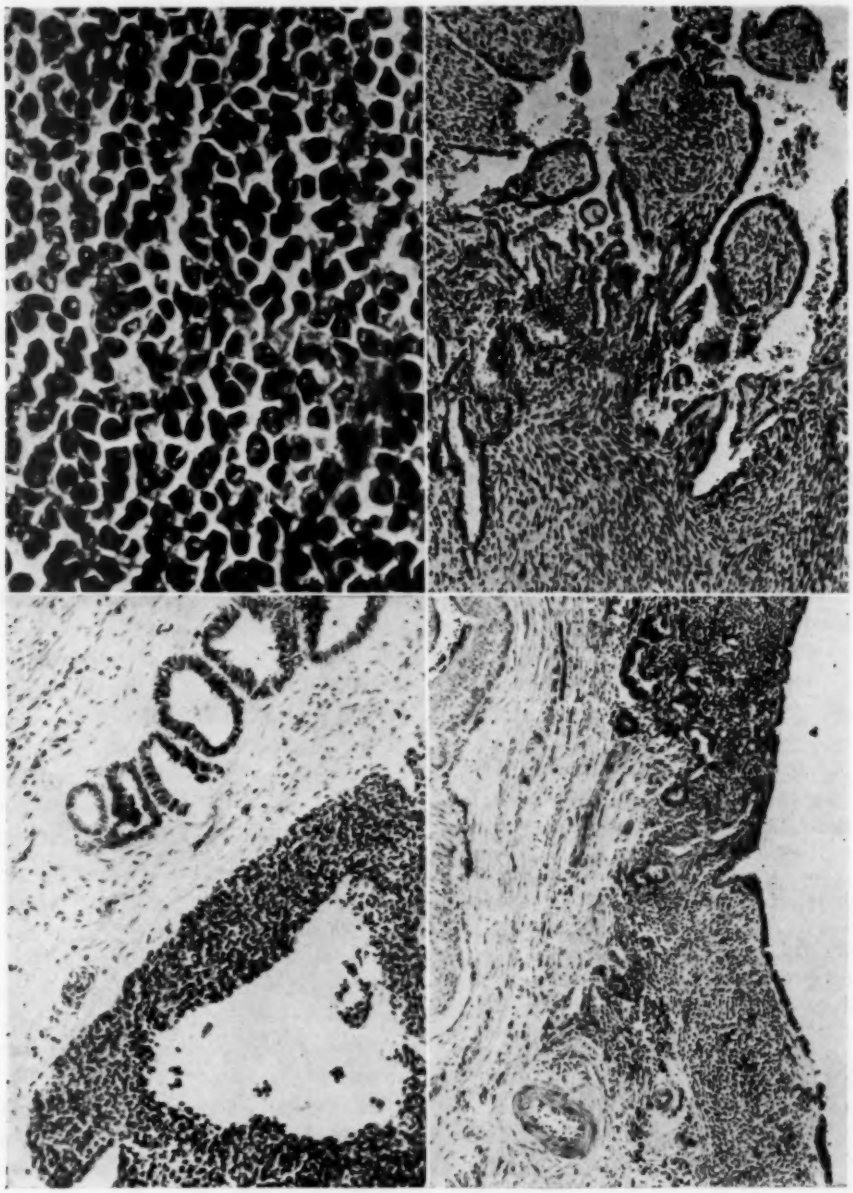

Fig. 1 (Case 1).—Upper left, high power photomicrograph showing detail of tumor cells: rounded, hyperchromatic nuclei surrounded by scanty cytoplasm, showing no specific growth pattern. Hematoxylin and eosin; magnification × 450.

Upper right, peritoneal surface of tumor showing papillary adenomatous growth of mesothelium over a sarcomatous base composed of spindle cells arranged in poorly formed bundles. Hematoxylin and eosin; magnification × 100.

Lower left, a nest of nondescript tumor cells in the parametrium adjacent to a "rest" of tubular structures lined by columnar epithelium, presumably Wolffian in origin. Hematoxylin and eosin; magnification × 115.

Lower right, serosa of Fallopian tube showing "atypical endometriosis" and its transition to an area of neoplastic transformation of subcoelomic mesenchyme. Hematoxylin and eosin; magnification × 85.

bowel. The inferior and anterior surfaces of the liver were studded with numerous plaques and nodules of tumor tissue and there were adhesions between these and the diaphragm. Posterior to the bladder a mass of similar tumor was found in the pelvis, adherent to the rectum and pelvic wall. Microscopic examination of numerous blocks of tumor tissue revealed an undifferentiated malignant neoplasm similar to that seen in the first surgical specimen. There was extensive necrosis of the tumor tissue taken from the recurrent pelvic mass as well as a pronounced radiation reaction at the operative site.

The final anatomic diagnoses were: (1) Postoperative state, recent, total hysterectomy for carcinosarcoma arising in the parametrium; (2) carcinosarcoma, recurrent in pelvis, sarcomatous component only, massive, with radiation reaction; (3) carcinosarcoma, metastatic to peritoneum (extensive) and liver (superficial), sarcomatous component only; (4) bronchopneumonia, confluent, extensive; (5) arteriosclerosis, generalized.

CASE 2.—F. L. (Boston Lying-in Hospital Cs-53-1, contributed by Dr. Craig W. Freeman, Minneapolis), a 43-year-old unmarried white woman, was admitted on Dec. 16, 1952, complaining of severe diffuse, lower abdominal pain which had been present for several days. She had experienced several similar episodes previously over the past year and had been hospitalized for a similar attack one month before admission. Her earlier attacks had subsided without specific treatment. Her abdominal pain was not accompanied by vaginal bleeding, urinary symptoms, disturbances in bowel function, or any systemic complaint. Pelvic examination at the time of hospitalization one month earlier failed to disclose any mass, but the accuracy of this examination was somewhat compromised by the fact that the patient was extremely tender at the time and full relaxation was not obtained. After a few days of symptomatic treatment during the present attack, pelvic examination was possible and disclosed the presence of a moderately large, ill-defined pelvic mass. It could not be determined whether the mass was uterine or adnexal in origin. Laparotomy was performed and the tumor was removed by morcellation. The neoplasm appeared to surround the greater portion of the corpus uteri. It was partly encapsulated and was adherent to the bowel. A few small nodules were present within the uterine wall compatible in appearance with leiomyomata. The ovaries and tubes were apparently separate from the tumor mass; one ovary contained a corpus luteum. In addition to the morcellated fragments of tumor, the uterus, cervix, and both tubes and ovaries were removed. On gross examination there was no evidence of tumor in the endometrium, endocervix, or exocervix. Histologic preparations were sent to Dr. Arthur T. Hertig in consultation. The patient was living and apparently well in June, 1954, 18 months after the operation.

Microscopic examination of sections through the tumor showed it to be composed of an even admixture of glands and stroma. The glands were lined by a single layer, occasionally pseudostratified, of columnar epithelial cells and resembled the tubular to moderately coiled glands of the endometrium. Some glands were lined by ciliated epithelium, while in others secretory activity in the form of subnuclear and, rarely, supranuclear vacuolation was present. A moderate number of glands contained secreted material within their lumina. An occasional mitotic figure was seen in a glandular cell, but no great degree of active proliferation was noted. There was no pleomorphism of the glandular component, nor did the pattern of glands suggest neoplasia. The glands were surrounded by a stroma which exhibited several different morphologic patterns; these patterns conformed to no detectible distribution, nor did they seem related to any specific state of the glandular epithelium in the vicinity. The least frequent pattern was that of fairly uniform, round to slightly ovoid cells, arranged in a reticulum resembling normal endometrial stroma to some extent, but mimicking even more closely the arrangement commonly seen in endometriosis. More frequently there was considerable pleomorphism and hyperchromasia of the stromal cells; in these areas the cells were larger than those mimicking endometriosis and mitotic figures were seen with ease in such areas. Occasional binucleate cells were noted, as well as cells with bizarre, irregular nuclei; atypical mitotic figures were also occasionally in evidence. Although the contrast between the non-neoplastic-appearing stroma and the neoplastic stroma was

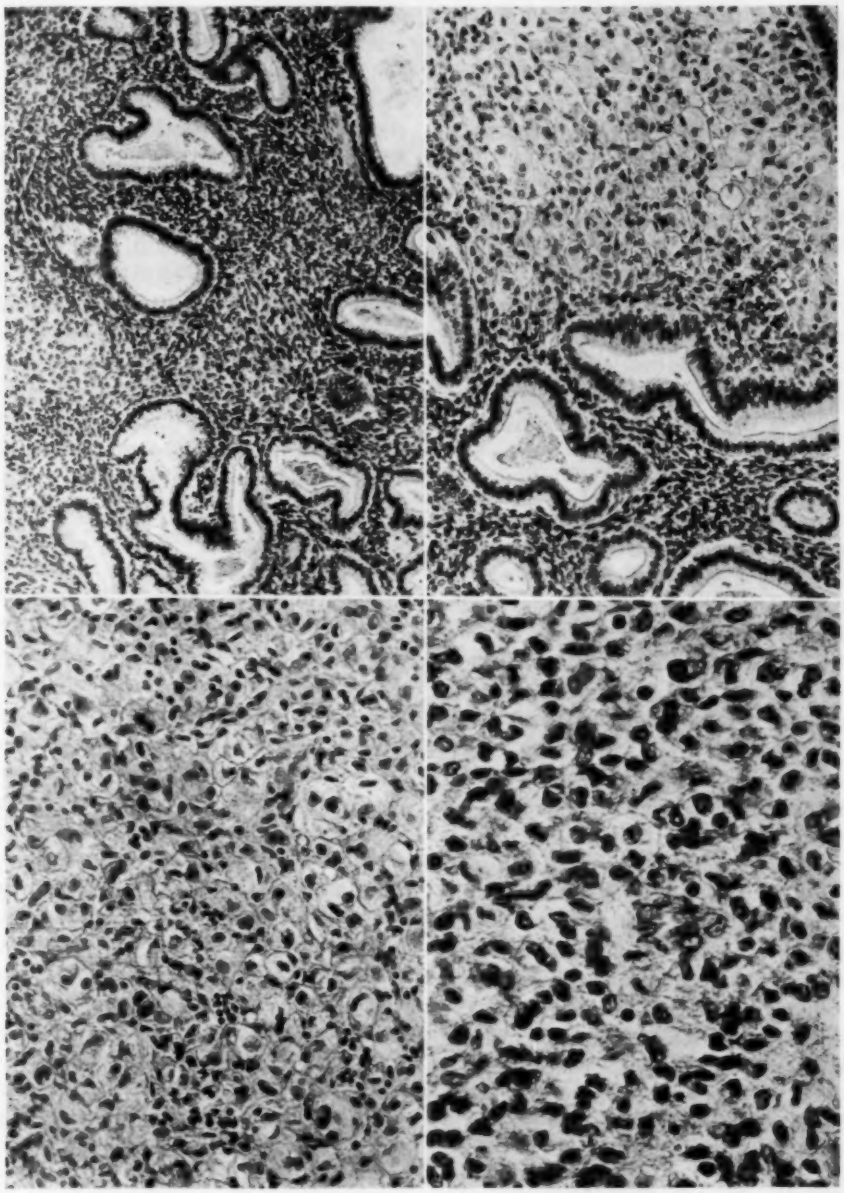

Fig. 2 (Case 2).—Upper left, endometriosis composed of non-neoplastic glands and an innocuous uniform cellular stroma. Hematoxylin and eosin; magnification × 100.

Upper right, endometriosis showing non-neoplastic glands and transition to a pleomorphic stroma composed of polyhedral cells with refractile interfaces and clear cytoplasm. Hematoxylin and eosin; magnification × 145.

Lower left, detail showing pleomorphic stroma of "decidua-like" cells. Hematoxylin and eosin; magnification × 200.

Lower right, detail showing sarcomatous stroma composed of smaller cells with dense hyperchromatic nuclei, variation in size and shape, and an occasional mitotic figure. Hematoxylin and eosin; magnification × 350.

quite striking, there were no abrupt changes noted; the transition was generally quite gradual.

Microscopic examination of the ovaries revealed them to be free from tumor. The endometrium was in the 17 to 18-day secretory stage. Microscopic examination of the nodules within the myometrium confirmed the gross impression that they were leiomyomata. The cervix showed moderate chronic cervicitis with a few Nabothian cysts. No evidence of tumor was seen in the corpus or cervix uteri or in the ovaries.

The final diagnosis was sarcomatous transformation of the stroma of endometriosis.

COMMENT

These two tumors exhibit several features in common as well as certain points of difference. In the first case, the predominant neoplastic element was sarcomatous; in the second case, the neoplastic element was entirely sarcomatous, the glandular element being benign. In each case the sarcomatous element was a poorly differentiated "round cell" sarcoma which resembled most closely sarcomas arising in the endometrial stroma. However, in neither case was there any alteration in the endometrium which could be construed as neoplastic; in fact, both tumors arose and remained outside of the corpus uteri, encasing it from the outside, and, in the first case, invading the external layers of the myometrium only superficially. Careful examination of the cervix, tubes, and ovaries failed to disclose a possible primary source in these organs. It is concluded that these two sarcomas arose from the pelvic supporting tissues which lie beneath the peritoneal surface.

In the first case, as the "round-cell" sarcoma encroached upon the peritoneal surface, it became admixed with a papillary tubular component, and there was a transition of the round-cell element to a spindle-cell form. In a restricted sense, this zone is a mesothelioma, as defined by Stout,* and the transition from normal to neoplastic patterns of mesothelial cells can be visualized; however, the tumor is predominantly mesenchymal. In this case, the presence of submesothelial neoplasm makes the transition less clear than in the classic demonstration by Rhind and Wright.[11] Although Maximow [12] and Stout and Murray [13] have demonstrated the ability of the versatile mesothelial cell to behave like a fibroblast when explanted, we do not feel that the major portion of the tumor in this case arose from transformed mesothelium. Not only is the general pattern unlike that seen in the recognized varieties of mesothelioma,[14] but, apart from the area near the peritoneum, there is no suggestion of a tubular or a fibrous pattern, nor an admixture of the two.[15] It is conceivable that the transition of the pelvic peritoneum from an indifferent, flat layer to an area of papillary neoplastic growth may represent an "induction phenomenon," the presence of the underlying sarcoma having stimulated the mesothelium to proliferate.

The presence of numerous tubular structures in the parametrium, closely admixed with the sarcomatous portion of the tumor, might serve to support the notion that the tumor arose from the stromal component of a vestigial remnant of the Wolffian apparatus. An alternative hypothesis might be presented to the effect that the sarcomatous portion of the tumor arose from the stromal component of endometriosis of the pelvic peritoneum and that the papillary epithelial-like element developed independently from a metaplastic pelvic mesothelium. In support of this hypothesis is the observation of foci of atypical metaplasia of the pelvic peritoneum associated with an atypical stromal response in areas which resembled bizarre endometriosis close to but separate from the tumor. While neither hypothesis is more attractive nor more susceptible of direct proof than the other, both imply that the tumor is derived from the subcoelomic mesenchyme.

In the second case, there can be little doubt that the tumor arose in the stromal component of pelvic endometriosis. It is of some interest that endometriosis was not present in the ovarian substance in either

* References 9 and 10.

case. The observation that, in the second case, many areas of the tumor resembled a decidua-like tissue is also of interest, for the endometrium itself was in the early part of the secretory (progestational) phase of the cycle, and secretory changes of a less degree were found in the glandular component of the endometriosis.

SUMMARY

Two cases of sarcoma probably arising in the subcoelomic tissue outside the internal female genital organs are presented. Despite evidence for a common histogenesis, these cases present contrasting clinical features and may have had different pathogeneses.

The first case arose in the parametrium and gave rise to extensive peritoneal spread, proving fatal five months after operation. Closely associated with the main tumor mass were non-neoplastic vestigial remnants of the Wolffian apparatus in the form of simple tubules. As the tumor encroached upon the peritoneum, it became admixed with a neoplastic papillary proliferation of mesothelial cells, permitting the tumor to be classified as a carcinosarcoma. Elsewhere along the pelvic peritoneum were areas of "atypical endometriosis."

The second case was a sarcomatous transformation of the stromal component of pelvic endometriosis. The patient was reported as living and symptom-free a year and a half after operation. Many of the neoplastically transformed cells were polyhedral with clearly defined cell margins and resembled decidual cells in some respects.

There was no source of primary tumor in the cervix or corpus uteri, tubes, or ovaries, in either case.

These tumors are thought to have arisen in mesenchymal tissue derived ultimately from the mesenchyme of the urogenital ridge. They are both classifiable as mesenchymomas and illustrate further the versatility of the subcoelomic mesenchyme.

Helpful suggestions were given by Dr. Arthur Purdy Stout and Dr. Hugh G. Grady who studied the cases and read the manuscript before publication.

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Cor Triatriatum
(Stenosis of Common Pulmonary Vein)

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The purpose of this paper is to report a case of a rare congenital cardiac lesion which so far has never been diagnosed clinically. Postmortem examinations have revealed this diagnosis approximately a dozen times. It seems that this type of anomaly, if recognized clinically, would be amenable to surgery.

REPORT OF A CASE

History.—The patient, a white boy 12 years of age, was admitted to the pediatric service of the University Hospitals with a chief complaint of heart and lung trouble. At the age of 3 years he had had a severe attack of whooping cough from which he never quite recovered. A few months later he was hospitalized for pneumonia. At the age of 5 years the patient was discovered to have blood in his sputum. In the course of the next seven years upper respiratory infections, pneumonia, and hemoptysis occurred quite frequently. Orthopnea, as well as exertional and nocturnal dyspnea, was present at the time of admission, and palpitations and tachycardia were usually associated. Episodes of cyanosis were also noted. A review of systems indicated that the patient complained about pain in his legs. The family history revealed that 1 sister had suffered from rheumatic fever, while the other 12 siblings and the parents were in good health.

Physical Examination.—This 12-year-old boy was markedly undersized and underweight, and there was much wasting of the quadriceps muscles. Examination of the lungs was essentially negative. Auscultation of the heart showed occasional extrasystoles and bursts of tachycardia. On percussion the heart seemed enlarged to the right. There was a Grade IV blowing systolic murmur of maximum intensity at the apex. The blood pressure was 96/40. The examination of the abdomen was negative. There was no clubbing of the fingers.

From the Department of Pathology, University of Wisconsin Medical School.

Laboratory Work.—Routine urinalysis was normal. Hemoglobin was 13.4 gm. per 100 cc., and red cell count was 4,850,000 per cubic millimeter. The white cell count was 6250 per cubic millimeter with a normal differential count. Corrected sedimentation rate was 12, and the hematocrit was 28.5%. Blood sugar and nonprotein nitrogen were normal. Chest x-rays showed left auricular enlargement and prominent pulmonary vessels. The electrocardiogram suggested right ventricular hypertrophy. On fluoroscopy the left atrium was enlarged and the right ventricle suggestively increased.

Course.—In the hospital the pertinent complication was frequent and severe aggravation of the chest condition. Dyspnea and occasional cyanosis remained unchanged. Digitalis and mercurials appeared to help until a sudden attack of pulmonary edema developed, and the patient died.

AUTOPSY REPORT (54:139 M. L.)

Heart.—The weight of the heart is 165 gm. The pericardium is smooth and glistening. There is a marked hypertrophy involving the right ventricle as well as the right and the left atria.

The right atrium appears moderately dilated, measuring 5×5×4 cm. The thickness of its wall is 2 mm. The auricle does not contain any thrombi. There are no pectinate muscles behind the terminal crest. The coronary sinus is guarded by the small Thebesian valve. At the opening of the interior vena cava there is a well-developed Eustachian valve. The fossa ovalis does not show any perforations. The foramina venarum are quite prominent.

The right ventricle appears markedly dilated, measuring 6×6×6 cm. The hypertrophic wall measures 1 cm. near its base and 0.6 cm. near its apex. The trabeculae and the papillary muscles are quite prominent. The conus arteriosus appears normal.

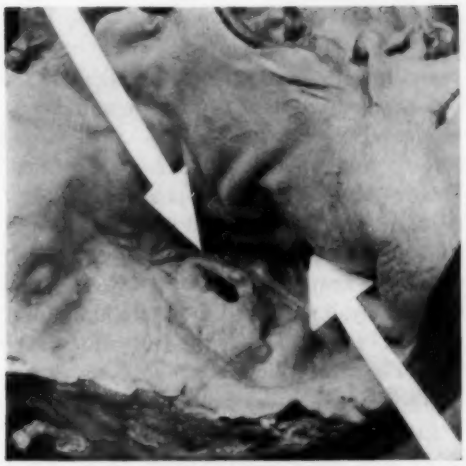
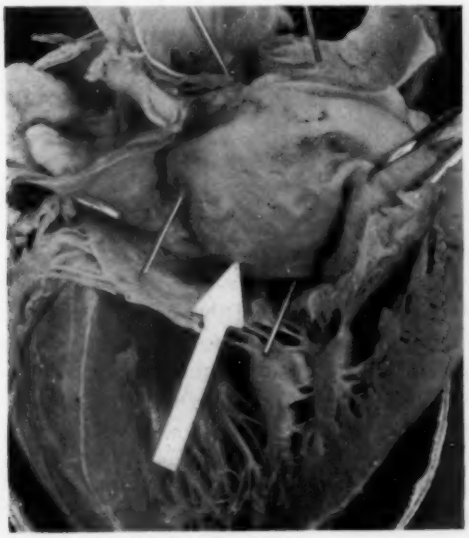


Fig. 1.—View from above into the common pulmonary vein. The dividing membrane, with arrows indicating the two perforations.

The tricuspid valve measures 9 cm. in circumference and slightly less than 3 cm. in diameter. All three cusps are well formed and show no evidence of adhesions. The chordae tendineae are not thickened or shortened.

The pulmonary valve measures 5 cm. in circumference. All three cusps appear normal.

Fig. 2.—View from below into the left atrium and ventricle. The arrow points to the dividing membrane. The probes pass through the two perforations. The mitral valve is normal.



The left atrium is divided by a horizontal membrane into an upper and lower compartment. The former is the larger and measures 5×5×5 cm. During removal, the upper compartment had been opened, so that it was impossible to determine the exact number and site of entrance of the pulmonary veins. The thickness of the muscular wall is 3 mm. The dividing membrane forms a dome downward and has two circular perforations measuring 0.6 and 0.5 cm. across. The membrane

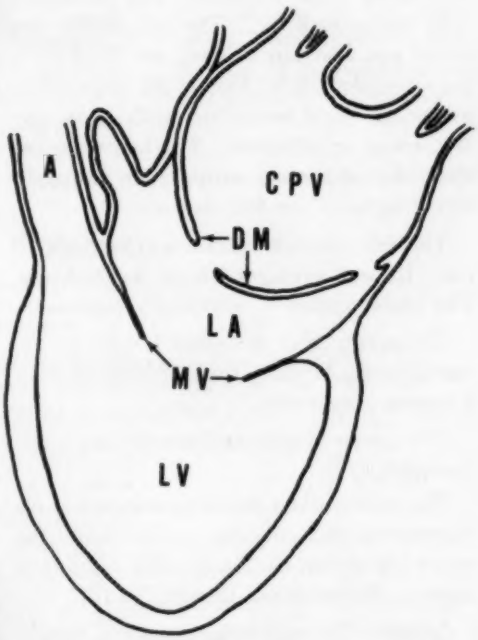


Fig. 3.—Diagram. *A* indicates aorta; *CPV*, common pulmonary vein; *DM*, dividing membrane; *LA*, left atrium; *MV*, mitral valve; *LV*, left ventricle.

itself is 5 cm. wide and 2 mm. thick. It is lined on both sides by a gray opaque thickened endothelium.

The lower atrium measures 5×4×2 cm. It communicates freely with the left auricle, which appears normal. The roof of the lower atrium is formed by the membrane. The largest spherical opening (0.6 cm.) is centrally located, while the smallest opening (0.5 cm.) is located in the periphery about 1 cm. above the posterior commissure of the

mitral valve. The floor is formed by the mitral valve. The lower atrium contains everything which normally is included in the left atrium. At first glance the membrane of the foramen ovale seems to be identified in the upper atrium. However, dissection reveals that we here are dealing with two membranes and that the wall of the pulmonary sinus is fused with the membrane of the foramen ovale.

The mitral valve is open for two fingers and seems to be sufficient. The circumference measures 8 cm. The two leaflets are about equal in size. They are lined by a smooth endothelium. Neither the cusps themselves nor their tendinous cords show any thickening or adhesions. The latter are not shortened and insert normally on the papillary muscles of the left ventricle.

The left ventricle measures 6.5×5.0×4.0 cm. Its wall averages 0.8 cm. in thickness. The trabeculation is moderately prominent.

The aortic valve measures 4.5 cm. in circumference. All three semilunar valves have a normal appearance.

The coronary ostia and arteries are unremarkable.

The endocardium shows no evidence of inflammation, and only the portion lining the upper left atrium and its dividing membrane appears thickened and fibrotic.

Lungs.—The right lung is enlarged, weighing 630 gm. The middle and lower lobes appear shrunken. In the upper lobe there are many small subpleural areas of hemorrhage.

The parenchyma has a consistency similar to muscle tissue. All arteries reveal sclerosis, and in some of them atheromatous plaques are seen.

The left lung weighs 630 gm. and has a similar appearance to the right.

Liver.—The liver weighs 900 gm. The surface is smooth. On cut surface the organ shows accentuation of the liver markings.

The remaining organs are essentially within normal limits.

MICROSCOPIC EXAMINATION

Heart.—The myocardial fibers of the left ventricle show a normal cross striation. There is a slight infiltration of the interstitial connective tissue by lymphocytes and phagocytes. No Aschoff's bodies can be identified. The endocardium appears normal.

In the left atrium there is marked thickening of the endocardium to more than 10 times its normal thickness. The connective tissue is hyalinized, and there are large focal deposits of calcium near the endocardial surface. The myocardium is composed of normal fibers. There is no evidence of inflammation.

Lungs.—The vessels are thickened, sometimes with obliteration of their lumen. All three layers show a marked proliferation of the connective tissue. However, the media and the adventitia seem to be more involved than the intima. Some of the vessels have been occluded, and there are several wedge-shaped infarcts in the pulmonary parenchyma.

Liver.—There are areas of fibrosis scattered throughout the liver, predominantly involving the areas around the central veins. Most of the liver cells appear normal except for those immediately surrounding the fibrotic areas, where some degree of atrophy is noted. It is believed this represents an early state of cardiac cirrhosis.

Diagnosis.—Cor triatriatum (common pulmonary vein).
Sclerosis, pulmonary vessels, extensive.
Cirrhosis, cardiac, slight.

COMMENT

Griffith [1] was the first to explain cor triatriatum as a failure of the common pulmonary vein to be incorporated in the left atrium. Hagenauer [2] suggested that an early closure of the opening might occur if the primitive pulmonary vein opened at an oblique angle into the distal atrium. Loeffler [3] did not accept Hagenauer's explanation and stated that the primary malformation was a disturbance of the normal growth of the posterior wall of the left atrium. Pfennig [4] assumed that a pri-

mary occlusion of the primitive pulmonary vein prevented it from being incorporated into the left atrium.

In this case the membrane of the foramen ovale was double owing to fusion of the pulmonary sinus to the true membrane. This fusion is caused by a marked dilatation of the common pulmonary sinus and might be explained by Pfennig's theory of a primary occlusion of the primitive pulmonary vein.

SUMMARY

An example of a triatrial heart has been described post mortem in a boy 12 years of age.

The malformation is apparently due to a failure of the common pulmonary vein to be incorporated into the left atrium. The dilatation of this vein seems best explained on the basis of a primary closure or constriction.

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News and Comment

ANNOUNCEMENT

Laboratory Refresher Courses of Communicable Diseases.—The Communicable Disease Center of the Department of Health, Education, and Welfare, Chamblee, Ga., will present a series of laboratory refresher training courses during the coming year as follows:

Dates	Courses No.	Courses	Duration, in Weeks
Sept. 12-23	8.40	Laboratory Diagnosis of Bacterial Diseases Part 1. General Bacteriology	2
Sept. 12-Oct. 7	8.00	Laboratory Diagnosis of Parasitic Diseases Part 1. Intestinal Parasites	4
Sept. 26-Oct. 7	8.41	Laboratory Diagnosis of Bacterial Diseases Part 2. General Bacteriology	2
Oct. 10-28	8.01	Laboratory Diagnosis of Parasitic Diseases Part 2. Blood Parasites	3
Oct. 17-28	8.20	Laboratory Diagnosis of Viral and Rickettsial Diseases	2
Oct. 17-28	8.50	Laboratory Diagnosis of Bacterial Diseases Enteric Bacteriology	2
Oct. 31-Nov. 4	8.26	Laboratory Diagnosis of Rabies	1
Oct. 31-Nov. 11	8.15	Laboratory Methods in Medical Mycology Part 1. Cutaneous Pathogenic Fungi	2
Nov. 14-25	8.16	Laboratory Methods in Medical Mycology Part 2. Subcutaneous and Systemic Fungi *	2
Nov. 14-25	8.55	Laboratory Diagnosis of Tuberculosis	2
Nov. 28-Dec. 9	8.17	Laboratory Methods in the Study of Pulmonary Mycoses	2
Dec. 12-16	9.40	Laboratory Diagnostic Methods in Veterinary Mycology	1
March 12-23	8.20	Laboratory Diagnosis of Viral and Rickettsial Diseases	2
March 26-30	8.26	Laboratory Diagnosis of Rabies	1
	8.05	Laboratory Diagnosis of Malaria †	2
	8.21	Virus Isolation and Identification Techniques †	2-4
	8.42	Typing of Corynebacterium diphtheriae †	1
	8.51	Special Problems in Enteric Bacteriology †	2
	8.52	Phage Typing of Salmonella typhosa †	1

* Completion of Course 8.15 or equivalent education or experience is a prerequisite.
† Courses offered by special arrangement only.

Information and application forms should be requested from Laboratory Training Services, Communicable Disease Center, U. S. Public Health Service, P. O. Box 185, Chamblee, Georgia.

Benign Chondroblastoma of Bone

An Unusual Localization in Temporal Bone

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and
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In 1931 Codman [1] described in detail the clinical and pathological features of a benign tumor, which he called epiphyseal chondromatous giant-cell tumor, located in nine instances in the upper end of the humerus. He recognized the benign nature of the neoplasm, although in four of the nine instances the lesion was overdiagnosed as a sarcoma by recognized authorities. Because of their epiphyseal location and the presence of giant cells, Codman believed they were a variant of giant-cell tumor. It was not until 1942 that Jaffe and Lichtenstein [2] presented a complete clinical and pathological study of nine additional cases and established the chondromatous nature of the tumor. Four of their cases were located in the lower epiphysis of the femur, three in the upper epiphysis of the tibia, one in the lower epiphysis of the tibia, and one in the tuberosities of the capital epiphysis of the humerus. In eight cases reported by Coley and Santoro,[3] the neoplasm was localized again in the long bones of the extremities. In the six cases reported by Hatcher and Campbell,[4] five tumors were located in the epiphysis of the long bones and one in the metaphysis. In 1949 Lichtenstein and Kaplan [5] reported an unusual localization of benign chondroblastoma in the femoral capital epiphysis, with extension into the neck of the femur. Copeland and Geschickter,[6] in 1949, reported 10 cases of benign chondroblastoma occurring in the long bones.

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This brief report is the only case found in the literature of a benign chondroblastoma of the skull.

REPORT OF A CASE

This patient was a 53-year-old white man who was admitted to the hospital with swelling of the right temporal region that had slowly increased in size during the past three or four months. Laboratory findings were not contributory. A roentgenogram of the skull (Fig. 1) showed a rarefied cystic defect of the lower portion of the parietal bone. Surgery done consisted only of curettage of the cystic lesion. Friable, grayish red and gritty tumor tissue was found.

Fig. 1.—Roentgenogram of cystic lesion of temporal bone.

Microscopically (Fig. 2) the tumor consisted of masses of uniform polyhedral cells, few spindle cells, multinucleated giant cells and focal areas of calcifying hyaline chondroid tissue, recent hemorrhage, and hemosiderin deposits, some within giant cells. Histological findings were those of a benign chondroblastoma, according to features presented by Jaffe and Lichtenstein.[2]

COMMENT

The variable microscopic picture has caused some difficulty in recognition of the tumor. The presence of an irregular calcifying matrix, giant cells, and masses of polyhedral cells are most commonly found.

The cartilaginous nature of the tumor is not obvious, since the polyhedral cells inter-

preted to be chondroblasts do not mature into recognizable chondrocytes. Hatcher and Campbell [4] reported two cases, out of a series of six, in which the cells were not even differentiated enough to produce the intercellular cartilaginous matrix. They believe that fibrosis and chondrofication represent an aging process in the tumor.

The majority of cases reported in literature have been found in young adults, although the age range has been from 8 to 60 years.

Conservative treatment consisting of surgical curettage is the recommended procedure, although radiation has also been used. Recurrences of the tumor have not been reported. Development of malignant tumor in bone following radiation is well known,* and it would seem unwise to use radiation in the treatment of a benign bone lesion.

The unusual localization of the tumor in this case demonstrates location need not be limited to epiphyseal site.

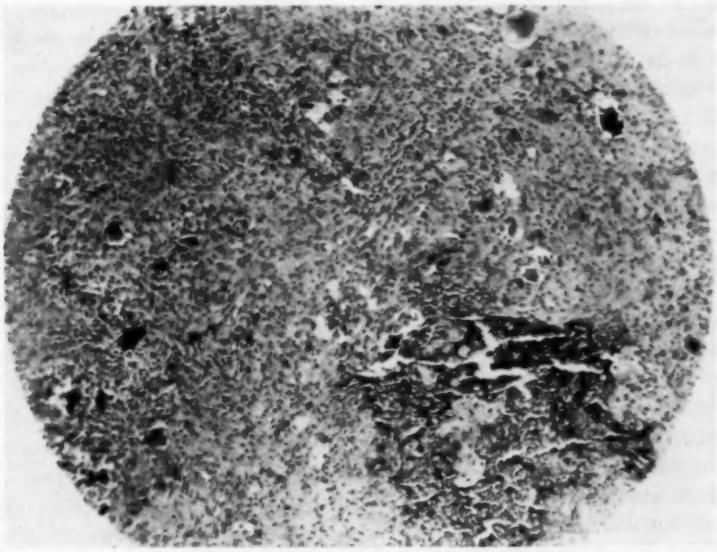

Fig. 2.—Photomicrograph of benign chondroblastoma tumor found in right temporal bone. Calcification is clearly seen in the lower right. Hematoxylin and eosin; reduced 3/10 from mag. × 80.

SUMMARY

As reported in the literature, benign chondroblastoma has been found chiefly in the extremities near epiphyseal centers. The case presented is the only one reported being found in the flat bones or skull.

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Serum Aldolase in Experimental Liver Necrosis

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The glycolytic enzyme aldolase was first demonstrated in the blood serum of rats in 1943.[1] It was observed then that the level of this enzyme in the blood serum was consistently higher in rats bearing certain large tumors than in normal rats. Employing an independent analytic method Sibley and Lehninger [2] some years later confirmed the earlier data and demonstrated that in these animals the high enzyme content of the serum was related to the presence of the neoplasm itself and not to any secondary effects of malignancy per se. Subsequently, it was shown [3] that the elevated serum-aldolase level was the result of an excessive liberation of the enzyme by the tumor mass, the rate of such liberation into the blood stream necessarily exceeding that at which it was normally eliminated from the circulation. It was suggested [3] that the source of the excess amounts of the enzyme was the continuous break-down of a large number of tumor cells, shown to be rich in aldolase. The numerous areas of necrosis always found on microscopic examination of tumor tissue constitute evidence for such increased cytolysis. Tissue-slice experiments demonstrated [3] that, under adverse environmental conditions such as the absence of sufficient amounts of oxygen, of adequate amounts of glucose, or of both, there was a prompt liberation of aldolase from either fresh slices of the Walker carcinosarcoma or from normal liver.

In a survey [3] of the aldolase content of the serums of 900 patients with a wide variety of diseases, the rather infrequent occurrence of abnormally high levels of the enzyme could be correlated with the presence of acute, extensive injury to some tissue. The pronounced rises in the enzyme level of patients with acute hepatitis, so in contrast to the normal or but slightly elevated levels in cases of obstructive jaundice or cirrhosis, suggest the clinical usefulness of such enzyme determinations as a diagnostic aid. Bruns and Puls [4] noted an elevated serum-aldolase level in patients with hepatocellular jaundice, and they reported an extreme elevation of the serum-enzyme values in mice following the intravenous injection of carbon tetrachloride.

The unusually high values of aldolase which occur in patients with hepatitis prompted our attempt to produce liver damage in the rat analogous to that seen in acute hepatitis in man. Such destruction of a functional tissue would provide a nonmalignant condition, characterized by necrosis, to further test the hypothesis that excessive cytolysis is the principal cause of elevated serum-aldolase levels.

The report of Kellner and Robertson,[5] including the observation that the intravenous injection of a solution of papain into rats resulted in selective necrosis of heart and skeletal muscle, suggested the use of this proteolytic enzyme as another means of producing injury to a tissue so that the possible subsequent changes in serum-aldolase content might be followed.

METHODS

All experiments were performed on rats of the Sprague-Dawley strain weighing 300 to 400 gm. The

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animals were maintained on a standard commercial laboratory ration.

Aldolase determinations were performed according to the method of Sibley and Lehninger [6] in which the triose phosphates resulting from enzymatic cleavage of fructose diphosphate are estimated colorimetrically. One milliliter of blood was obtained from the heart while the animals were under ether anesthesia; 0.1 ml. or less of serum is required for the assay. Repeated determinations could be performed on the same animal.

Liver damage was produced in one group of animals by the inhalation of the vapor of carbon tetrachloride. The animals were placed in a sealed chamber through which air was drawn after passing through carbon tetrachloride warmed in a water bath. Definite liver changes were produced by a six-hour exposure and an air flow of 4 liters per minute, which vaporized 102 ml. of carbon tetrachloride per

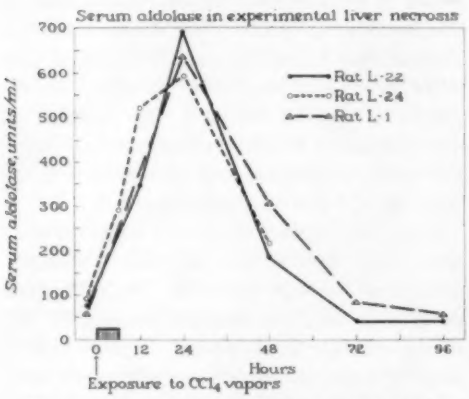


Fig. 1.—Serum-aldolase level in rats with focal hepatic necrosis produced by the inhalation of the vapors of carbon tetrachloride.

hour. Blood was drawn for the determination of serum aldolase and of total serum bilirubin immediately after exposure and at varying intervals of time thereafter. Representative animals were killed at these times; the livers were removed, fixed in 10% formalin, and appropriately sectioned and stained for histologic examination.

Five per cent solutions of crude, commercial papain (Nutritional Biochemicals Corp.), prepared in isotonic saline solution and made slightly alkaline to facilitate solution, were injected into the femoral veins of a second group of rats. Serial determinations of the serum aldolase and of the serum bilirubin were made at varying intervals after injection. Animals were killed at different time intervals and their livers, hearts, diaphragms, and other tissues were fixed and prepared for histologic study.

Solutions of crystalline papain (Mann Research Laboratories), with and without the addition of cysteine to serve as an activator, were also injected intravenously into a series of rats and similar determinations were made. The relative proteolytic activity of these preparations was determined by a modification of the pepsin assay method of Anson.[7]

Obstructive jaundice was produced in a fourth series of rats by the ligation of the bile duct. Triple ligation with wide separation of the cut ends of the duct was necessary to prevent a prompt reestablishment of bile flow.

RESULTS

The normal serum-aldolase levels of the rat range from 40 to 100 units per milliliter. Immediately after the six-hour exposure to carbon tetrachloride vapors, the aldolase level in the serum greatly increased and ranged from 200 to 300 units per milliliter. These values continued to rise, reaching levels 24 hours after exposure to the fumes, which ranged from 600 to more than 1000 units per milliliter.

A prompt fall in the enzyme level of the serum then occurred at a rate which was similar to that of the initial rise, so that normal values were again restored 48 to 72 hours after the exposure period. The animals did not appear ill and did not show jaundice or biliuria. Their total serum-bilirubin values remained normal (0.2 mg. per 100 cc. or less). The results of representative experiments on three rats are shown in Fig. 1.

The degree of cellular damage in the livers of these animals was found to parallel the rise in serum aldolase. Immediately after the exposure to the fumes of the drug, when increased values of aldolase were already disclosed, areas of cloudy swelling and hydropic degeneration were apparent in the liver. At 24 hours after exposure there were extensive areas of complete necrosis of the liver cells. These foci were chiefly central in location and they often involved more than two-thirds of each lobule. But at 72 hours there was usually no evidence of necrosis, and the only abnormality seen was a minimal round-cell infiltration. Fibrosis or derangement of the lobular architecture was not encountered in any animal after a single, or even once-repeated, exposure to carbon tetrachloride vapor. Repeated exposure of the same animals to the

same amount of the drug resulted in a repetition of this pattern of serum-aldolase elevation and histologic liver changes. The maximal enzyme values reached were then often higher, but they returned to normal levels in about the same time interval following exposure (Figs. 2, 3, and 4).

Following the intravenous injection of 0.5 ml. of the 5% solution of crude papain, there was an immediate rise in the serum aldolase, reaching a maximal value of 1000 to 1500 units per milliliter at 24 hours after injection. Decreased values were recorded after this time, and normal levels were restored 72 hours after the injection. Crude papain induced very obvious changes in the physical condition of the animals. There was definite jaundice, biliuria was detectable, and increased levels of serum bilirubin were recorded. The foci of necrosis observed in the liver after administration of papain were similar to those which followed the inhalation of carbon tetrachloride vapor, although they were present to a more marked degree in the case of papain than in that of carbon tetrachloride (Fig. 5). We did not observe necrosis of the heart or skeletal muscle; however, small areas of hemorrhage were seen in these tissues and the other organs studied. Impaired coagulation of the blood was apparent, as evidenced by protracted bleeding after any incision and lengthened clotting time. Injection of smaller amounts of crude papain, as 0.2 to 0.4 ml. of the 5% solution, had a similar although less pronounced hepatotoxic action, while the injection of more than 0.5 ml. usually resulted in the death of the animal, apparently as a result of extensive visceral hemorrhages. The activity of a solution of crude papain was rapidly lost on standing, for after 24 hours, even if the solution was kept in the cold, the foregoing effects were not produced on intravenous injection.

Following the injection of a solution of crystalline papain in doses representing up to 10 mg. of the enzyme, there were no apparent changes in the rats; alterations in the normal serum-aldolase levels, or histologic changes in the livers or other tissues, did not occur. A short incubation of the enzyme solution with cysteine before injection did not result in a potentiated action. Estimation of the proteolytic activity of these solutions showed that 10 mg. of crystalline papain had more than twice the ability to digest hemoglobin as did 0.5 ml. of a 5% solution of crude papain. Since cysteine interfered with the assay procedure, any increased activity from its addition was not measured.

After the ligation of the bile duct, the animals were jaundiced and showed biliuria, and progressively increasing bilirubinemia developed. In most animals changes in the serum-aldolase level did not occur, while in some a slight rise was recorded on the first or second day after ligation, with a subsequent return to normal values (Fig. 6). A definite bleeding tendency was observed in these animals.

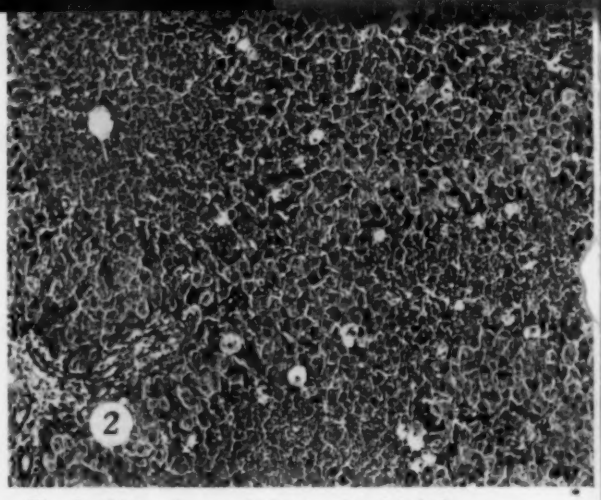
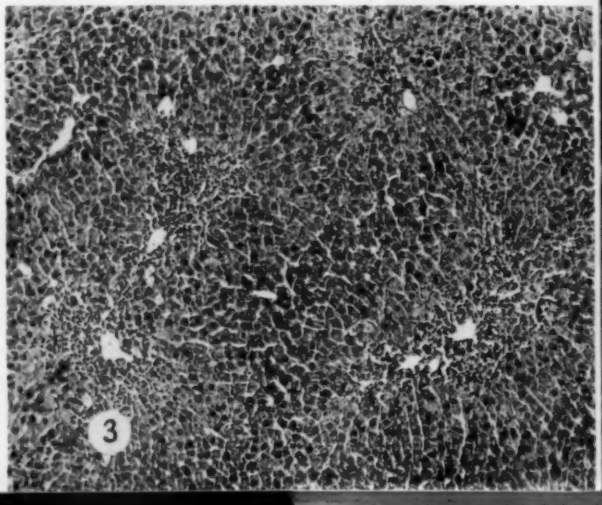


Fig. 2.—Liver of a rat 24 hours after inhalation of carbon tetrachloride vapors, showing acute focal necrosis. Serum aldolase then 700 units per milliliter. × 75.

Fig. 3.—Liver of a rat 48 hours after inhalation of carbon tetrachloride vapors, showing early regeneration and round-cell infiltration. Serum aldolase had then fallen to 200 units per milliliter. × 75.



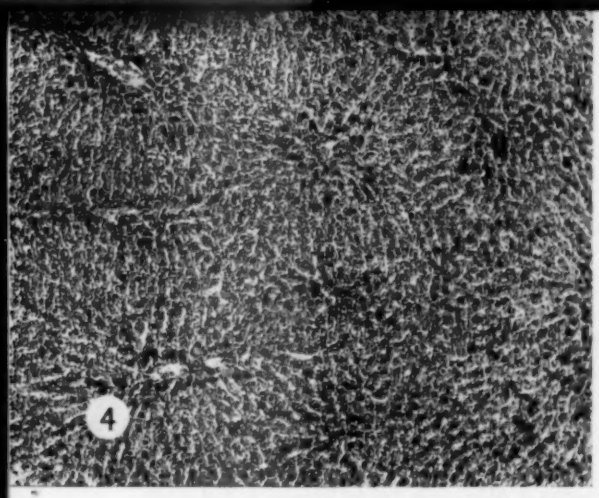


Fig. 4.—Liver of a rat 72 hours after inhalation of carbon tetrachloride vapors, showing almost complete regeneration. Serum aldolase had then returned to normal level. × 75.

COMMENT

The changes observed in the livers of rats following carbon tetrachloride intoxication closely paralleled the pathologic changes of severe acute hepatitis in man. Likewise, the variations in serum-aldolase level were very similar. In both the experimentally induced and the clinical cases a prompt marked rise in the enzyme content occurred, followed by a rapid return to normal values. These elevations in aldolase could be correlated with the appearance and the extension of the hepatic necrosis. With the cessation of active cellular destruction, the excessive liberation of the enzyme decreased, and the serum level was brought back to normal through some mechanism of elimination.

These experimental studies confirmed the clinical observation that obstructive jaundice does not produce an alteration in the serum-aldolase level. As studies on patients with liver disease of various types had suggested,[3] elevation in serum aldolase cannot be related to bile retention, as is the case with serum alkaline phosphatase.

The hepatic necrosis produced by the intravenous injection of crude papain resulted in similar changes in aldolase levels. The biologic effects of this substance, as shown by these experiments, were, however, quite unlike those reported by Kellner and Robertson.[5] We noted focal hepatic necrosis as the primary lesion, together with a hemorrhagic tendency when larger doses were given; but any direct action on either cardiac or skeletal muscle was not observed. Experiments with crystalline papain suggest that these effects are not due to the proteolytic action of the crude preparation. The nature of the toxic principle, however, was not determined.

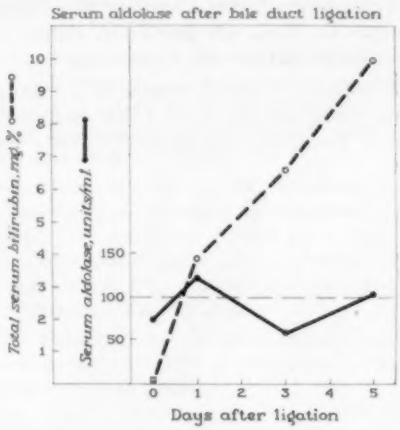
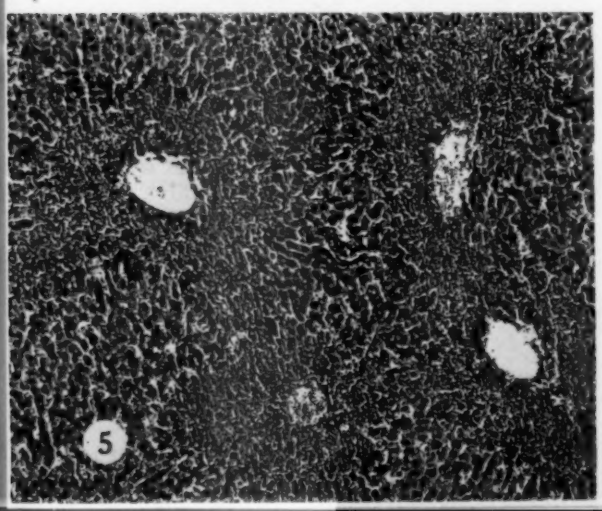


Fig. 6.—Serum-aldolase level and the total serum bilirubin after ligation of the bile duct in the rat.

Fig. 5.—Liver of a rat 24 hours after intravenous injection of crude papain solution, showing marked acute focal necrosis. × 75.



CONCLUSIONS

The production of focal hepatic necrosis in rats by the inhalation of carbon tetrachloride vapor resulted in a pronounced but transient elevation of the serum-aldolase content.

The histologic changes in the liver and the variations in the serum-aldolase levels induced by carbon tetrachloride were compar-

able to those changes in these categories observed in acute hepatitis in man.

Intravenous injection of a solution of crude papain produced similar liver changes and also elevation of the serum aldolase, but such effects were not reproduced by the injection of a solution of crystalline papain of equal proteolytic activity.

Experimental obstructive jaundice in the rat did not alter the serum-aldolase level.

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Inhibition of Experimental Atherosclerosis by Sulfated Alginic Acid

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In a previous publication from this laboratory,[1] evidence was presented that heparin will prevent the development of atherosclerosis in rabbits force-fed cholesterol. Our findings were in agreement with the claim of Graham and co-workers,[2] and they were subsequently substantiated by Horlick and Duff.[3]

Since heparin is a sulfated polysaccharide, we sulfated various macromolecular sugars and screened them for antilipemic properties in the rat. Of several such heparin-like compounds tested, sulfated alginic acid displayed the highest antilipemic activity (equal to that of heparin), with negligible anticoagulant and no hemorrhagic effects.[4]

The present paper deals with the effects of this synthetic antilipemic agent on the atherosclerosis and other phenomena produced by cholesterol feeding in rabbits.

MATERIALS AND METHODS

1. General Experimental Arrangement

Twenty littermate pairs of New Zealand White rabbits (10 male and 10 female pairs) were divided in two groups of 20 animals each, each rabbit of one group being represented by its littermate partner in the other.

Throughout the duration of the experiment, both groups were pair-fed rabbit pellets that contained 1.2% cholesterol (Nutritional Biochemicals) and 5% cottonseed oil in addition to their normal components, except on Sundays, when normal rabbit feed was given. The high-lipid feed was prepared according to our specifications at the factory of Buckerfield, Ltd., Vancouver.

After two weeks on the high-lipid diet, the 15 pairs that developed the highest and most uniform lipemia and hypercholesteremia were selected for further experimentation, whereas the remaining 5 pairs were discarded.

Starting on the 15th day of cholesterol feeding and continuing to the end of the experiment, every animal of one group was injected twice daily with 5 mg/kg. sulfated alginic acid (prepared as described elsewhere [4]). This dosage was raised to 10 mg/kg. after the 35th day.

The experiment was terminated 60 days after the commencement of cholesterol feeding with the killing and autopsy of all animals.

2. Measurements of Aortic Atherosclerosis

Aortic atherosclerosis was evaluated in every animal with a morphological (total atheroma area) and a chemical (total cholesterol content) criterion.

The total atheroma area was obtained by indirect planimetry as follows: Each aortic tube was removed from the valves to the iliac bifurcation and slit longitudinally. Transparent paper tracings of the outlines of all visible atheromatous lesions and of the whole aortic ribbon were made. The tracings of the lesions were cut out carefully, weighed in a microanalytical balance, and compared with the weight of the tracing of the whole ribbon. The amount of atherosclerosis found in each aorta was thus expressed as the percentage of the total aorta area occupied by atheromata.

The cholesterol content of each aortic ribbon was determined by stripping off the adventitia, cutting the intima-media in small pieces, and extracting them with acetone-alcohol in Soxhlet assemblies, after weighing. The materials of each aorta were extracted twice for four hours, and the two extracts were pooled and subjected to quantitative cholesterol analysis, by the method of Sperry and Webb.[5]

From the Anatomy Department, University of British Columbia.

3. Blood Data

Blood samples were taken repeatedly from the ear veins of all animals, with a 26 gauge needle. Plasma was obtained by centrifugation of blood mixed 10:1 (v/v) with 0.1 M sodium citrate.

Plasma turbidity was recorded as the logarithmic scale reading of the 1:10 diluted plasma in a Klett-Summerson colorimeter, against a water blank, using a red filter.

Plasma total cholesterol concentration was determined in accordance with the technique of Sperry and Webb.[5]

Blood clotting time was estimated by tipping whole blood samples in conical centrifuge tubes every 30 seconds until clotting occurred.

4. Other Data

Body weights were obtained at various intervals. Records of liver, spleen, and left adrenal weights were made. Paraffin sections of the spleen were cut at 7μ and stained with hematoxylin and eosin. The cholesterol concentration of the liver was determined as above.[5]

5. Statistical Evaluation of Quantitative Data

The standard error, ϵ, of each mean was calculated from the formula $\sqrt{\frac{\Sigma d^2}{n^2 - n}}$; the t value, from the formula $\frac{\bar{X}_1 - \bar{X}_2}{\sqrt{\epsilon_1^2 + \epsilon_2^2}}$, and the p value, from Fishers' tables of t.

RESULTS

1. Plasma Turbidity (Table 1)

As usual, cholesterol feeding caused a progressive rise of plasma turbidity which corresponded to the gradual accumulation of light-scattering macromolecular lipoproteins and chylomicrons in the blood (cf. Pierce and Bloom [6]). At the start of the experiment all plasmata were clear (with colorimeter readings of less than 12 units), but as the alimentary lipemia developed they became more and more turbid.

Sulfated alginic acid (SAA), started on the 15th day at twice 5 mg/kg. daily, reduced the lipemic turbidity significantly. Doubling the dosage of SAA (after the 35th day) resulted in complete suppression of lipemic turbidity.

The antilipemic effect of SAA at twice 10 mg/kg. daily was highly significant statistically ($p < 0.01$), and it was clearly visible by naked-eye observation. In the last two samplings, the plasmata of all SAA-treated animals were clear, whereas those of all untreated controls were milky.

2. Plasma Cholesterol (Table 1)

Cholesterol feeding produced a rapidly rising hypercholesteremia, resulting in a 20-fold increase of the normal plasma cholesterol level at the end of the experiment.

Table 1.—*Plasma Lipemic Turbidity, Plasma Cholesterol, Blood Clotting Time, and Body Weights (Means ± Standard Errors)* *

Days After Commencement of Cholesterol Feeding	Untreated Controls (Cholesterol Feeding Only)	Sulfated Alginic Acid Treated (Cholesterol Feeding + SAA Injections)	Statistical Significance of Difference (p)
Plasma lipemic turbidity, logarithmic colorimetric scale readings			
0	7.6 ± 0.6	7.7 ± 0.7	> 0.9
9	31.4 ± 6.8	31.8 ± 6.9	> 0.9
14	39.3 ± 6.3	41.7 ± 6.5	0.8
21	31.6 ± 5.9	15.9 ± 3.7	0.05-0.02
34	44.4 ± 6.6	17.2 ± 2.5	0.02-0.01
41	46.4 ± 7.9	6.8 ± 0.7	< 0.01
51	60.0 ± 9.5	11.0 ± 1.7	< 0.01
Plasma total cholesterol, mg./100 cc. plasma			
0	76.5 ± 9.9	64.2 ± 13.8	> 0.9
9	709.2 ± 111.0	744.7 ± 105.6	> 0.9
21	1089.2 ± 160.2	961.9 ± 94.2	0.5-0.4
41	1210.0 ± 134.0	613.0 ± 45.2	< 0.01
51	1570.0 ± 182.0	593.0 ± 26.5	< 0.01
Blood clotting time, minutes			
30	3.98 ± 0.21	3.75 ± 0.45	0.7-0.6
51	3.52 ± 0.33	4.39 ± 0.62	0.3-0.2
Body weights, kg.			
0	2.35 ± 0.06	2.39 ± 0.05	0.7-0.6
60	2.98 ± 0.05	2.56 ± 0.05	< 0.01

* Sulfated alginic acid injections were started on the 15th day after commencement of cholesterol feeding at twice daily 5 mg./kg. The dosage was doubled on the 35th day.

SAA treatment did not influence the hypercholesteremia at twice 5 mg/kg. daily, but when this dosage level was doubled it caused a highly significant drop of the blood cholesterol concentration to less than half the untreated control value.

3. Blood Clotting Time (Table 1)

SAA failed to affect the blood clotting time at either dosage level. The differences between the mean clotting times of the SAA-treated and untreated control animals were not significant.

TABLE 2.—*Findings in Aorta, Liver, Spleen, and Adrenal (Mean Values ± Standard Errors)*

		Untreated Controls	Sulfated Alginic Acid Treated	Statistical Significance of Difference (p)
Aorta	Area occupied by atheromata, %	14.1 ± 2.96	3.5 ± 1.17	< 0.01
	Total cholesterol content, mg./aorta	4.94 ± 0.90	2.46 ± 0.38	0.02-0.01
	Weight, mg./kg. body wt.	277.0 ± 19.3	308.0 ± 16.8	0.3-0.2
Liver	Weight, gm./kg. body wt.	47.4 ± 4.45	49.1 ± 1.12	0.8-0.7
	Cholesterol content, mg./gm. liver	26.8 ± 3.57	24.0 ± 3.70	0.7-0.6
Spleen	Weight, gm./kg. body wt.	0.50 ± 0.06	3.64 ± 0.79	< 0.01
Adrenal	Weight, mg./kg. body wt.	61.7 ± 2.7	59.5 ± 4.6	0.7-0.6

4. AORTIC ATHEROSCLEROSIS (TABLE 2)

SAA inhibited the development of atherosclerosis as judged by both the morphological and the chemical criterion. The average aorta of the SAA-treated animals presented only one quarter the atheroma area and one half the cholesterol content of the untreated control aorta. Both effects were highly significant statistically.

Since atheromata are known to consist of several other lipids (triglycerides, phospholipids, etc.) in addition to cholesterol, it may not be surprising that the development of morphological lesions was inhibited more than the deposition of cholesterol in the aortic wall.

It should be noted that there was no significant difference between the aortic weights of the two groups.

5. SPLEEN (TABLE 2, FIGS. 1 AND 2)

SAA caused a pronounced enlargement of the spleen (to seven times the size of the control organ). The surface and the cut section of these enlarged spleens were of an intense yellow color. Histologically, the entire red pulp was occupied by large lipid-filled mononuclear cells ranging in structure from the foam to the signet-ring-cell type. Although most of these lipophages seemed to be tightly packed between the sinusoids, foam-cells were also encountered free within the lumen of blood vessels. In addition, the parenchyma contained some focal infiltrations of eosinophilic leucocytes. Since the lymphatic elements of the white pulp seemed to be normal in bulk and structure, and since

Fig. 1.—Typical spleen of a cholesterol-fed rabbit (untreated control). Note the numerous atheromatous arterioles in the otherwise normal parenchyma. Hematoxylin and eosin; low power.

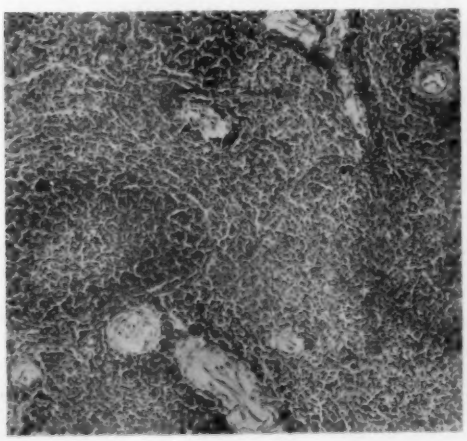
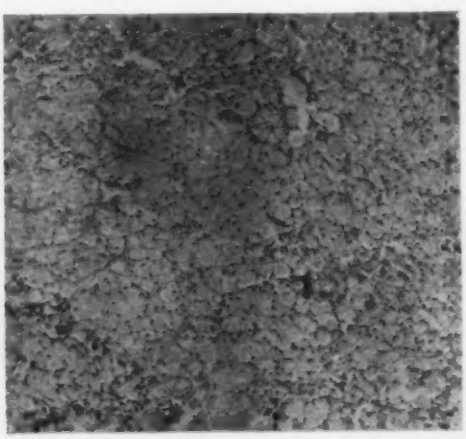


Fig. 2.—Typical spleen of a cholesterol-fed and SAA-treated rabbit. Note the complete replacement of the normal parenchyma by very large, tightly packed, lipid-filled cells. No atheromatosis was encountered in the splenic arterioles of this group. Hematoxylin and eosin; low power (magnification identical with that in Figure 1).



there was no hyperemia or trabecular thickening, this splenomegaly appeared to be due to the extraordinary accumulation of large lipid-loaded macrophages in the white pulp. The whole picture resembled, in many respects, the splenic findings of some human reticuloendothelial lipidoses.

The spleens of the untreated controls were of normal gross and microscopical appearance except for the presence of atheromatous arterioles in most of them.

No atheromatous arterioles were observed in the sections of the spleens of the SAA-treated group.

Karyorrhexis was absent from the white pulp of both groups.

6. Liver (Table 2)

SAA had no effect on the weight or the cholesterol content of the liver, but the livers of all SAA-treated animals were of a yellowish color, whereas those of all untreated controls were brown.

7. Adrenal (Table 2)

There was no difference in weight between the adrenals of the two groups.

8. General Remarks

SAA retarded significantly the growth of the animals to which it was given. In 60 days, the untreated controls increased their body weight by 26.9%, while the SAA-treated rabbits showed an increment of only 7.1% (Table 1). Autopsy revealed that the animals of the SAA-treated group had less omental, mesenteric, and retroperitoneal fat than the untreated controls.

Three animals died early in the course of the experiment (one in the control, two in the SAA-treated group) of intestinal impaction. Daily supplementation of 1 gm. dried alfalfa leaves in the diet of every rabbit prevented the recurrence of such accidents.

Throughout the duration of the experiment, all animals displayed normal spontaneous activity and appeared to be in good clinical condition.

COMMENT

Since SAA, like heparin, abolished the lipemia, reduced the hypercholesteremia, and suppressed the development of atherosclerosis in cholesterol-fed rabbits, it is probable that antiatherogenic activity is shared by all sulfated polysaccharides with strong lipemia-clearing activity. Since, however, SAA did not prolong the blood clotting time, it can be concluded that anticoagulant properties are not an essential prerequisite of antiatherogenic action by sulfated polysaccharides.

In further contrast to heparin,[1] SAA retarded body growth, it did not affect the adrenal weight, and it caused massive lipid storage in the spleen.

The growth-retarding effect of SAA could not have been responsible for its antilipemic and antiatherogenic action. In the first place, the full antilipemic effect of SAA can be obtained within an hour of subcutaneous injection.[4] Furthermore, contrary to previous assumptions, recent investigations by Duff and his collaborators[7] demonstrated that failure to gain weight and even marked body weight loss do not inhibit the development of lipemia, hypercholesteremia, or atherosclerosis in cholesterol-fed rabbits. Duff's findings have been extended by Goldner and co-workers,[8] who have reported that inanition increases the lipemia and atherosclerosis in cholesterol-fed rabbits, and by Katz and co-workers,[9] who found that starvation retards the regression of coronary atherosclerosis in cholesterol-fed chicks.

The absence of an effect of SAA on the adrenal weight would indicate that the inhibition of adrenal enlargement observed early in the course of heparin treatment[1] was not causally related to its antiatherogenic action. The same fact, together with the absence of lymphatic karyorrhexis, also speaks against the possibility that SAA had strong "stressor" properties (cf. Selye[10]).

The fact that SAA did not affect the liver cholesterol concentration suggests that this compound did not interfere with cholesterol absorption from the intestine but that it acted at the blood-tissue level.

The finding of massive lipid storage in the spleens of the SAA-treated animals is, at the moment, compatible with any one of the following hypotheses:

1. SAA stimulates intense lipophagic activity in reticuloendothelial cells.

2. SAA transforms the blood lipids in a way that greatly facilitates their phagocytosis (and removal from the blood) by reticuloendothelial cells.

3. SAA mobilizes lipids from other tissues of the body and makes them available to the same cells.

Since no microscopic examination of the reticuloendothelial elements in organs other than the spleen was made in this experiment, future studies will have to show whether SAA affects the reticuloendothelial system as a whole or whether its effect is limited to the splenic portion of this system.

It is now well known that heparin attacks alimentary lipemia indirectly, i. e., by activating a lipolytic enzyme system in the body, the so-called "lipemia-clearing factor." * The actions of this factor on lipemic blood consist of a rapid breakdown of "giant" lipoprotein molecules (with high Sf rate) to small molecules (with low Sf rate),[2] of a drop in chylomicron count and size,† and of fat hydrolysis.‡ To these effects, which can be obtained both in vivo and in vitro, one may add the reduction of the blood cholesterol concentration, which so far has been reported only in vivo.§ Since the lipemic turbidity of the plasma is proportional to its content of large light-scattering lipoprotein molecules, the heparin-induced clearing of lipemia reflects the disappearance of these macromolecular units from the bloodstream.

It thus seems justified to attribute the antiatherogenic effect of heparin to the production of "lipemia-clearing factor," and, since SAA shares with heparin the ability to produce this factor,[4] it might be assumed that SAA inhibits alimentary atherosclerosis through the same mechanism. The marked reticuloendothelial response to SAA indicates, however, that this compound acts through a more complex mechanism, involving perhaps stimulation of reticuloendothelial phagocytosis in addition to production of "lipemia-clearing factor."

It is of interest to note that inhibition of alimentary atherosclerosis combined with marked reticuloendothelial lipidosis has been reported in rabbits treated intravenously with sorethytan (20) monooleate (Tween 80) by Payne and Duff.[17] Nevertheless, the fact that Tween 80 increased the hypercholesteremia (instead of decreasing it, as SAA did) speaks against the possibility that SAA acted partly through the same mechanism as Tween 80.

Systematic studies of the SAA-induced reticuloendothelial reaction and of its relationship to the antiatherogenic effect of SAA are now in progress.

SUMMARY

Sulfated alginic acid, a synthetic heparin-like compound, abolished the lipemia, reduced the hypercholesteremia, and inhibited the atherosclerosis produced by cholesterol feeding in rabbits, without prolonging the blood clotting time. This substance also caused a marked enlargement of the spleen and massive lipid storage in the reticuloendothelial cells of that organ.

This study was supported by the National Research Council of Canada and the Banting Research Foundation. Dr. A. J. Wood, of the Animal Nutrition Department of the University of British Columbia, cooperated in this study.

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† References 11 and 14.

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News and Comment

ANNOUNCEMENTS

Course in Medical Mycology.—A course in medical mycology is to be offered from July 5 to July 30, at Duke University School of Medicine and Duke Hospital. It will emphasize the clinical, pathologic, and therapeutic aspects of fungous infections. Patients, clinical materials, cultures, and laboratory animals will be available for study. An opportunity to study gross and microscopic pathologic materials will be given to those qualified by interest and previous training. Practical laboratory aids that help to establish a definitive diagnosis will be stressed. The course is open to clinicians, pathologists, bacteriologists, technicians, and others who have an interest in the medical phases of mycology. Classes will meet six days a week. Inquiries should be directed to Norman F. Conant, Duke Hospital, Durham, N. C.

GENERAL NEWS

Wisconsin Society of Pathologists Elect Officers.—At the recent annual meeting of the Wisconsin Society of Pathologists the following officers were elected for the ensuing year:

President: M. B. Llewellyn, M.D.

Vice-President: Norbert Enzer, M.D.

Secretary-Treasurer and Councilor to A. S. C. P.: Robert S. Haukohl, M.D.

Delegate to State Medical Society: Gorton Ritchie, M.D.

Alternate Delegate: Robert S. Haukohl, M.D.

Board of Censors: Etheldred Schafer, M.D., S. B. Pessin, M.D., and Walter H. Jaeschke, Ch.

A Round Table discussion on "Air Embolism" was conducted by Dr. Charles P. Larson, Tacoma, Wash. Dr. Larson also spoke at a session of the State Medical Society on "Investigation of the Unknown Dead."

CORRECTION

In the review of "Gerichtliche Medizin," by Dr. Gerhard Hansen, in the March, 1955, issue of the ARCHIVES (p. 395) the name of the publisher was incorrectly given. This book is published by VEB Georg Thieme, Hainstrasse 17-19, Leipzig C 1, Germany.

The Effect of Parathyroidectomy on the Evolution of Hypervitaminosis A in Rats

Histological and Chemical Observations

JONATHAN COHEN, M.D.
CHARLOTTE L. MADDOCK, M.D., Boston
and
S. BURT WOLBACH, M.D.†

Changes in the skeleton of weanling rats given excessive amounts of vitamin A form a pattern of acceleration of certain normal sequences of bone formation, the mechanism of which remains unknown. It has been suggested [1] that this pattern, including, as it does, acceleration of the endochondral, appositional, and resorptive aspects of bone formation, resembles a response to hormonal stimulation because of its integrated character. The present study was designed to determine whether the pattern of response was altered if the parathyroid glands of the rats were ablated prior to the induction of hypervitaminosis A.

EXPERIMENTAL DATA

The experimental animals were 39 male rats of Wistar strain, which were 22 to 23 days old and weighed 37 to 58 gm. Thirty were parathyroidectomized by electrocauterization * on the 25th or 26th day of life, and 3 or 4 days were devoted to their recovery from the procedure. Vitamin A ‡ was administered starting on the 29th or 30th day and was given by gavage six times a week, the dose level being either 1000 I. U. per gram of body weight or 100 I. U. per gram of body weight. Two diets were used, differing merely in the amount of calcium carbonate incorporated, the levels being 1.3% and 0.5%, respectively. The composition of the diet was as follows:

	Per Cent
Casein (vitamin-free, Labco brand)	24
Dextrose	64
Salt mixture [2]	1
Potassium acid phosphate	2
Peanut oil	8
Calcium carbonate	1.3 or 0.5

The diets contained 6.24 gm. of phosphorus per kilogram of diet and, for the normal and high calcium variations, 6.9 gm. and 10.1 gm., respectively, of calcium per kilogram of diet. The vitamin supplements per kilogram of diet were 30,000 units of vitamin A and 5,000 units of vitamin D incorporated in the peanut oil, and brewer's yeast 50 mg/kg. of diet. The special diets were begun four days prior to parathyroidectomy and were continued until time of killing.

The rats were divided into the following categories:

Group A. Intact diet controls

Group B. Parathyroidectomized diet controls

Group C. Intact rats given 1000 units of vitamin A per gram of body weight

Group D. Parathyroidectomized rats given 1000 units of vitamin A per gram of body weight

Group E. Parathyroidectomized rats given 100 units of vitamin A per gram of body weight

The rats were kept in individual cages with wire bottoms in a well-ventilated, thermostatically controlled room. Water and food were supplied ad libitum.

Except for four animals that died unexpectedly, the rats were killed by exsanguination under ether

Aided by grants from the Nutrition Foundation and the Williams-Waterman Fund for the Combat of Dietary Diseases.

From the Laboratories of Nutritional Research of the Division of Laboratories and Research and the Department of Orthopedic Surgery at The Children's Medical Center and The Children's Cancer Research Foundation.

† Dr. Wolbach died March 19, 1954.

* This procedure was carried out with the assistance of Dr. Roy O. Greep, Dr. Paul Munson, and Mr. Wolfgang Toefel, Harvard Dental School.

‡ Supplied by the Hoffmann-LaRoche Company as a synthetic palmitate ester containing 1,000,000 I.U./gm., which was diluted 1:5 in peanut oil for greater accuracy in administration.

anesthesia, and postmortem examination was done on each animal. The tissue techniques were those used in previous studies.§ In addition to the histological sections of the knee joints, the tissues surrounding the upper trachea and larynx were dissected en bloc, and serial blocks were sectioned and studied for persistence of remains of the parathyroid glands. In only two instances were such remnants found, and in neither of these were the remaining cells numerous or hyperplastic.

The studies of the blood ‖ were as follows: Note was made of the coagulation time, hematocrit value, and sedimentation rate, and the centrifuged supernatant serum was examined for clotting and gross lipemia. Serum mucoprotein was determined by a modification of Winzler's method, serum calcium by Sobel and Sobel's method,[3] serum phosphorus by the Fiske and SubbaRow method, and serum protein by either the Kjeldahl or the falling-drop technique.

RESULTS

Parathyroidectomy caused a significant mortality in those animals that received the diet containing normal amounts of calcium, namely, 2 of 10 animals; whereas, in the group of 18 that received a high-calcium diet, none succumbed during the first few days postoperatively. After the immediate postoperative period had elapsed, the behavior of all parathyroidectomized animals was one of increased excitability, but no tetany was observed. This behavior apparently was not affected by the administration of vitamin A at either dose level, except for the changes attributable to inanition or vitamin A toxicity. The gain in weight of parathyroidectomized animals was considerably below that of intact animals in any of the groups studied. Those parathyroidectomized rats that received vitamin A at the dose level of 100 units per gram of body weight followed essentially the same weight curve as parathyroidectomized dietary controls, but those receiving 1000 I. U. gained considerably less during the first few days and then showed the typical inanition previously described.

The changes caused by hypervitaminosis A in parathyroidectomized animals under either of the two dietary regimens were indistinguishable from those in intact animals. Once differences in total dose, due to differences in weight and duration of survival, were taken into account, no influence attributable to parathyroidectomy could be discovered either in the degree of inanition or in the skeletal, testicular,[4] or hematological[5] changes caused by hypervitaminosis A. These changes have been described in detail in previous communications and the descriptions need not be repeated at length. A short summary of them therefore is given below.

At the dose level of 1000 units a day per gram of body weight the age in days at time of killing varied between 33 and 47 days, all the animals being moribund when killed. They were anemic and showed pallor of eyes, ears, viscera, and muscles. Their hematocrit values ranged between 10% and 25% and their erythrocytes sedimented readily, leaving a cloudy lipemic supernatant serum that clotted only after prolonged standing. Hemorrhages were found in nearly all the animals, particularly in the epididymis and retroperitoneally, but also, on occasion, in the pericardial or pleural spaces, in the thymus gland, myocardium, lungs, muscles, and cranium. The lymph nodes were markedly hemorrhagic.

The changes in the skeleton at the 1000-unit dose level were those of acceleration of consumption of the epiphyseal cartilage and acceleration of remodeling. The characteristic deposition of immature endosteal bone in large amount, replacing some of the compact bone in the diaphysis, was readily observable. The accelerated remodeling progressed to fracture in only one parathyroidectomized animal, however, and in none of the intact animals. The testicular changes, consisting of degeneration of the seminiferous tubules, were seen in all animals that lived beyond 39 days of age.

At the dose level of 100 units per gram of body weight daily the changes in the skeleton were seen to a less degree than at the higher dose level, but in this group one fracture also was noted. The hemorrhagic tendency was more in evidence, coordinate

§ References 1 and 2.

‖ Mrs. Antoinette Curry, of the Clinical Laboratory, Children's Medical Center, made many of these determinations.

Effects of Parathyroidectomy and Hypervitaminosis A on Serum Concentrations of Calcium, Phosphorus, Protein, and Mucoprotein

Category and No. Rats								
Group	No.	Pa *	Vit. A Dose, I. U.	Age at Killing, Days	Serum Calcium, Mg.%	Serum Phosphorus, Mg.%	Serum Protein, Gm.%	Serum Mucoprotein, Mg. Tyrosine %
An †	(2) †	0	0	39-40	9.8 (2) † ±0.20	8.4 (2) ±0.55	4.6 (2) ±0.55	5.8 (2) ±0.06
Ah †	(2)	0	0	39-40	9.8 (2) ±0.15	8.4 (2) ±0.40	5.2 (2) ±0.50	6.3 (2) ±0.70
Bn	(6)	+	0	38-135	5.9 (4) ±0.85	11.7 (4) ±0.88	5.8 (4) ±0.30	4.9 (6) ±0.72
Bh	(6)	+	0	30-72	5.8 (4) ±0.38	15.6 (3) ±2.83	5.8 (4) ±0.30	5.5 (4) ±0.23
Cn	(3)	0	1000	39-40	7.8 (2) ±0.05	7.8 (2) ±0.55	3.7 (1)	10.3 (2) ±0.06
Ch	(4)	0	1000	39-46	8.4 (4) ±0.63	6.2 (3) ±0.13	4.5 (3) ±0.27	16.3 (4) ±3.74
Dn	(7)	+	1000	33-47	5.6 (6) ±0.93	10.9 (5) ±1.84	2.9 (1)	9.6 (5) ±1.90
Dh	(4)	+	1000	44-47	5.6 (3) ±0.62	10.4 (3) ±0.58	3.9 (3) ±1.02	15.5 (4) ±1.59
E	(5)	+	100	57-133	5.9 (4) ±0.45	11.0 (4) ±0.10	5.5 (4) ±0.83	9.0 (3) ±1.26

* Parathyroidectomized animals.
† *n* refers to the group of animals on the diet normal in calcium content (1.0% calcium); *h* refers to those on the diet high in calcium (0.69% calcium). The figures in parentheses refer to the number of animals or chemical determinations in the group.

with the longer period of administration of the vitamin and with the increased maturity of the animals. Testicular changes also were quite evident in this group in all the animals concerned.

The results of the chemical determinations on the serum are given in the accompanying Table. They demonstrate a significant lowering of the serum calcium level in all parathyroidectomized animals with a concomitant rise in serum phosphorus. Vitamin A at either dose level had no apparent effect on these determinations. Values for calcium in individual animals, however, showed wide variation. The values for serum phosphorus were much less variable. Although the numbers of determinations of these values in the various experimental groups were too few for statistical reliability, there was no indication that the age of the animals after parathyroidectomy, or the two diets used, influenced the level on the variation of the serum calcium or phosphorus. The intact animals showed no significant change in serum phosphorus or calcium attributable to vitamin A.

The serum protein was appreciably lowered in those animals that received vitamin A, particularly where survival was so prolonged that general toxicity due to the vitamin was evident. The serum mucoprotein, expressed as milligrams of tyrosine per 100 ml. of serum, was considerably elevated in these animals, whether expressed in that fashion or as percentage of a total protein; but no apparent relationship between the degree of lowering of serum proteins and the amount of elevation of serum mucoprotein content was demonstrable. The variability in the results of analyses of both serum protein and serum mucoprotein in those animals given vitamin A, whether parathyroidectomized or intact, was great. In those animals not receiving vitamin A most of the values were well within the normal limits and the variability was considerably less.

COMMENT

The primary object of these experiments was to determine whether or not ablation of the parathyroid glands of weanling rats had any effect on the evolution of the skeletal changes induced by hypervitaminosis A. No difference in reaction to excessive dosage of vitamin A was detected between parathyroidectomized and intact rats, thus demonstrating conclusively that the parathyroid glands have no part in mediating the effect of vitamin A on the skeleton. The hemorrhagic syndrome and the testicular changes caused by hypervitaminosis A in adolescent or adult rats also were not affected by parathyroidectomy.

Having recorded these negative findings, it seems of greater interest to take up in this discussion the data obtained on the control parathyroidectomized animals, since relatively few studies are available on the effect of parathyroidectomy alone on the growth of parathyroidectomized rats. Shelling[6] has made the statement that rats may be raised to maturity after parathyroidectomy, but his data on these animals remain unpublished. In confirming this statement we wish to point out that the weight gain of these parathyroidectomized rats did not keep pace with that of intact controls on the same diet, and the animals, while healthy in appearance, showed uniform hyperexcitability. Histological sections of the skeleton during the period of observation, which reached a maximum of 113 days, showed no significant deviations from normal in the sequences of bone formation in the parathyroidectomized group.

Greep and Fischer¶ have studied the effects of parathyroidectomy on rats in relation to the content of calcium and phosphorus in the diet. For their animals, which were followed for four weeks, similar weight curves were recorded as for our animals, when diets of approximately the same content of calcium and phosphorus were used. The range of concentrations of serum calcium and serum phosphorus in our experiments corresponds to their unpublished observations except for the following detail. In their experiments, the "blood levels of calcium and phosphorus showed a marked tendency to reflect the relative concentration of these substances in the diet." With the two diets used, our data disclosed a consistent deviation in the concentration of calcium or phosphorus in the serum, the one being consistently low with a wide variation in values, while the other was consistently high with only slight variation. We must point out, however, that the two diets used did not vary as widely in their calcium and phosphorus content as those of Greep and Fischer, which may explain this slight variation in results.

The lack of effect of hypervitaminosis A on the concentrations of serum calcium and phosphorus confirms the findings of Davies and Moore.[8] The fall in serum protein and rise in serum mucoprotein, while not specific, have been present consistently in these, as well as in previous, experiments with hypervitaminosis A in intact rats. The fall in serum protein may be due to a combination of factors, liver damage and hemorrhage being the most prominent. The rise in serum mucoprotein is thought to be related to the dissolution of skeletal tissue resulting from increase in remodeling and the concomitant break-down of other connective tissues resulting from general inanition.

¶ Greep, R. O., and Fischer, C. J.,[7] and personal communication.

SUMMARY

Parathyroidectomy did not change the evolution of hypervitaminosis A in weanling rats. The effects of parathyroidectomy alone on the growth of bone and on serum concentrations of calcium and phosphorus are recorded.

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Pathologic Reactions Associated with Bite of Latrodectus Tredecimguttatus

Observations in Experimental Animals

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Bites of the Latrodectus tredecimguttatus spider have presented an important medical problem in the Istrian districts of Yugoslavia.[1] The experiments here reported were made on cats, rabbits, guinea pigs, rats, and mice which had been bitten on the nose by this type of spider. Histopathologic examinations were made on animals which died or were killed at varying intervals after the bite. Effects were noted in the liver, kidneys, spleen, lymph nodes, brain, thymus, and adrenal gland. The most striking changes were seen in the liver and kidneys, the intensity depending on the duration and, probably, on the quantity of poison absorbed.

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In the liver, from 30 minutes to 6 hours after the bite the hepatic cells became swollen, with the process gradually increasing so that 10 hours after the bite there was dissociation of cells, massive hyperemia, and pericapillary edema. Necrobiosis became evident within 12 hours (Fig. 1), followed within 24 hours by larger areas of lobular necrosis (Fig. 2).

In the kidneys degeneration of the tubular epithelium ensued within 10 to 20 hours, with definite evidence of necrosis at 24 hours. In two cases (rats) in which death occurred after 40 hours, thrombosis of capillaries was present.

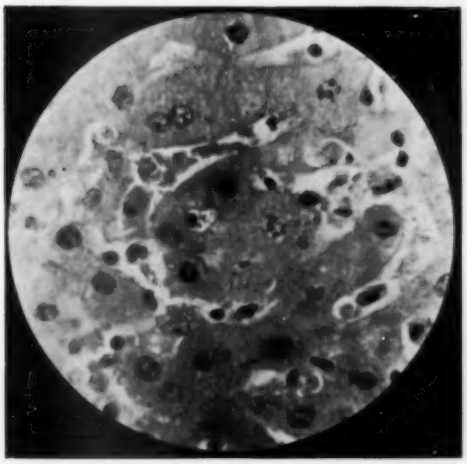

Fig. 1.—Necrobiosis of the epithelial cells in the liver of the rat; reduced ⅔ from mag. × 420.

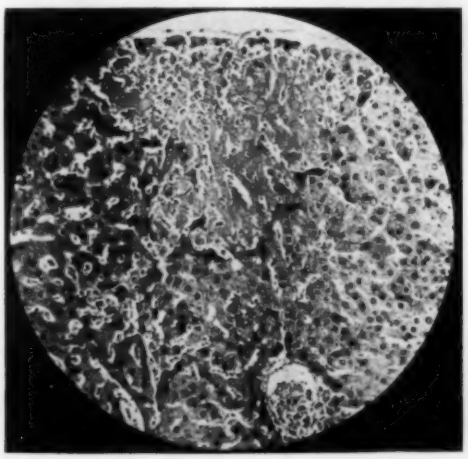

Fig. 2.—Necrosis in the liver of the rat; reduced ⅔ from mag. × 270.

The spleen and lymph nodes underwent early hyperemia, followed by evidences of lymphoid depletion, marked lymphorrhexis, and hemosiderosis (Fig. 3). The thymic cells showed marked pyknosis and karyorrhexis within 12 hours, and the reticulum cells became swollen and prominent. The cells of the zona fasciculata in the adrenals became swollen, vacuolated, or finely granular, followed in 24 hours by disorganization of the columns (Fig. 4). Perivascular hemorrhages were noted in the brains and hearts in some animals. Pulmonary edema was present in many instances.

Experiments * using vital staining with trypan blue indicated that the poison was able to pass across the blood-brain barrier.

* References 2 and 3.

Fig. 3.—Lymphopenia in the spleen of the rat; reduced ⅙ from mag. × 270.

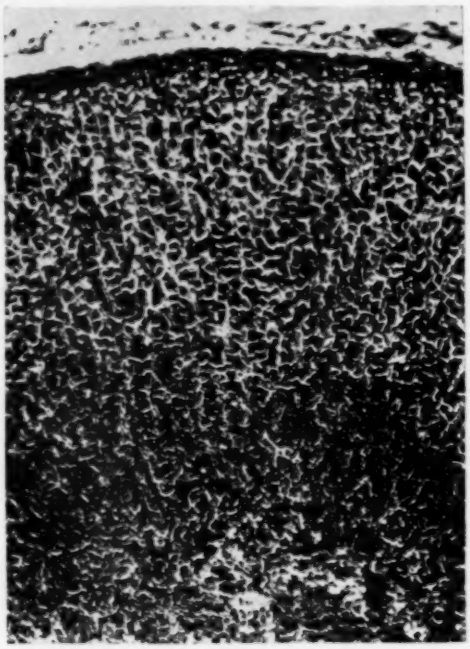

Fig. 4.—Dissociation of the cells of the cortex in the adrenal gland of the rat; reduced ⅙ from mag. × 270.

SUMMARY

The toxic injury produced by the poison of this spider is essentially a parenchymatous necrosis affecting blood vessels and epithelial cells in particular, as well as lymphoid cells and nervous tissues. These effects may be an expression of the consequences of a general adaptation syndrome.

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Glucose Antagonists in Experimental Cancer

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In our previous papers,* we stressed the fact that the antagonists of essential metabolites, although exerting an antitumor activity, have only a limited inhibitory effect on experimental malignant tumors. Our present investigation concerns two antagonists of glucose: 2-desoxy-D-glucose (2 DG) and galactose. It was presumed that the antagonists of glucose might interfere with the formation of the glycosidic ring of deoxyribonucleic acid (DNA) and might impede the multiplication of malignant cells by inhibiting their glycosis. It is well known that tumor tissue, in contrast with most normal tissues, possesses the ability to glycolyze glucose at a higher rate both aerobically and anaerobically.[5]

2-Desoxy-D-glucose is, by its chemical structure, an analogue of glucose. It differs from glucose by the absence of one oxygen atom at the second carbon atom. Cramer and Woodward [6] have shown that 2 DG competes with glucose in the yeast fermentation system and thereby inhibits fermentation of glucose. It was also demonstrated by Woodward and Hudson [7] that 2 DG inhibits the anaerobic and aerobic glycolysis of glucose, as well as fructolysis, by slices of rat tumors. It does not significantly affect the endogenous respiration of the tissues, normal or malignant, in concentration at which glycolysis is inhibited. The type of inhibition appears to be competitive, since inhibition is reversed by increasing glucose in the tumor slices.

Galactose is antagonistic to glucose, according to Wooley,† although its competitive action is not defined and is less clear than that of 2 DG. Galactose does not inhibit the fermentation of glucose by yeasts, according to Lauchenauer,[8] but apparently it moderately inhibits glycosis in tumor tissue.[9] Magyar [10] and Moseley and Chornock [11] found that the galactose concentration in blood is depressed by previous administration of glucose. Neither fructose nor rhamnose has such a hindering effect. The analysis of the voluminous literature on galactose suggests that it is not a true antimetabolite of glucose but exerts certain antagonistic activity towards it.

MATERIAL AND METHOD

Crocker rat carcinoma, a very virulent tumor, was used for these experiments. This tumor never recedes, and it kills animals in 22 to 28 days. At that time, the tumor size is 12 to 16 cm.[3] The rats were of a British brown-white strain, BCC, originally obtained from the Cancer Institute of Columbia University. The animals were kept on an exclusive meat isocaloric diet, which they tolerate well. This diet did not affect the tumor growth. The antagonists were added to the rations in various amounts from 2% up to 20%. The tumor was transplanted three days after the animals were placed on their respective diets, and tumor size was measured every seven days.

Blood glucose was determined by Folin-Malmrose technique, and blood galactose concentrations were estimated by the yeast fermentation method described by Lauchenauer.[8]

2-DESOXY-D-GLUCOSE

Eighty rats, average weight 193 gm., were divided into four groups. Group I, 20 rats, served as control and were kept on an exclusive meat diet. Groups

From A. P. Cooke Memorial Cancer Laboratory, Florida Southern College.

Aided by grants from The Marcia Tucker Foundation and The Maxine Ford Ellis Cancer Fund.

* References 1 through 4.

† Personal communication to the authors.

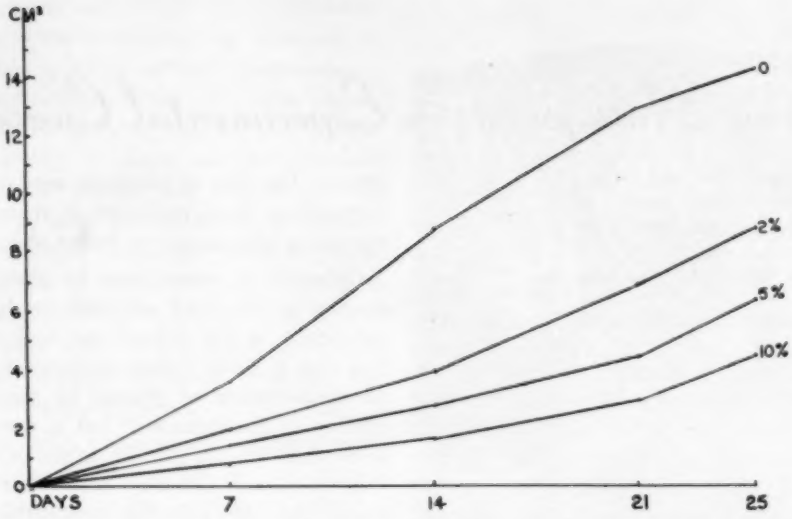

Chart 1.—Effect of 2-desoxy-D-glucose on Crocker rat carcinoma. All rats were on an exclusive meat isocaloric diet. Each group contained 20 rats. Group I, control. Group II, 2% of 2 DG; Group III, 5% of 2 DG; Group IV, 10% of 2 DG. Average tumor size is shown in cubic centimeters.

II, III, and IV, of 20 rats each, were placed on a meat diet containing 2%, 5%, and 10% of 2 DG, respectively.

Chart 1 summarizes the results so obtained.

The figures of Chart 1 clearly indicate the tumor activity of the antimetabolite under the present conditions of experimentation. The most effective dose was that of 10% added to meat ration. At the end of four weeks the control animals were all dead, while the treated animals remained alive, with tumors four times smaller than those of the control rats. Instead of 14.3 cm.³ average tumor size for control, Group IV averaged 4.5 cm.³). There was, however, no complete regression of tumors except in two instances. The rats of all three treated groups eventually died from cancer. The average span of life for the four groups was as follows: Group I (control), 27 days; Group II (2% 2 DG), 41 days; Group III (5% 2 DG), 55 days, and Group IV (10% 2 DG), 63 days. Thus the rats on a diet containing 5% or 10% of 2 DG lived about two times longer than the controls.

The chronic toxicity of this antimetabolite was evidenced only when 10% or more of it was added to the daily ration. The rats on a diet containing 10% of 2 DG lost weight, up to 20% of their original weight during the four-week period. From the average weight of 197 gm., the rat weight in Group IV was reduced to 163 gm. There was no weight loss in Groups II and III after six weeks of dieting.

2 DG affected the glucose blood concentrations. Table 1 gives the average figures of glucose blood concentrations of the four groups of rats.

Apparently, the antimetabolite induced a moderate hypoglycemia when given in sufficiently large amounts. This fact should be taken into consideration when estimating the antitumor activity of 2 DG. However, as our

TABLE 1.—*Effect of 2-Desoxy-D-Glucose on Blood Glucose Concentrations* *

	Blood Glucose Concentrations, Mg./100 Cc., Av.			
	9 A. M.	1 P. M.	4 P. M.	8 P. M.
Group I, control	75	128	115	84
Group II, 2% 2 DG	65	88	74	66
Group III, 5% 2 DG	57	78	62	57
Group IV, 10% 2 DG	45	66	55	38

* Rats were kept on an exclusive meat isocaloric diet. Feed was given at about 10 a. m. and was consumed in 30-45 minutes. The blood samples were taken at 9 a. m. and at 1, 4, and 8 p. m.

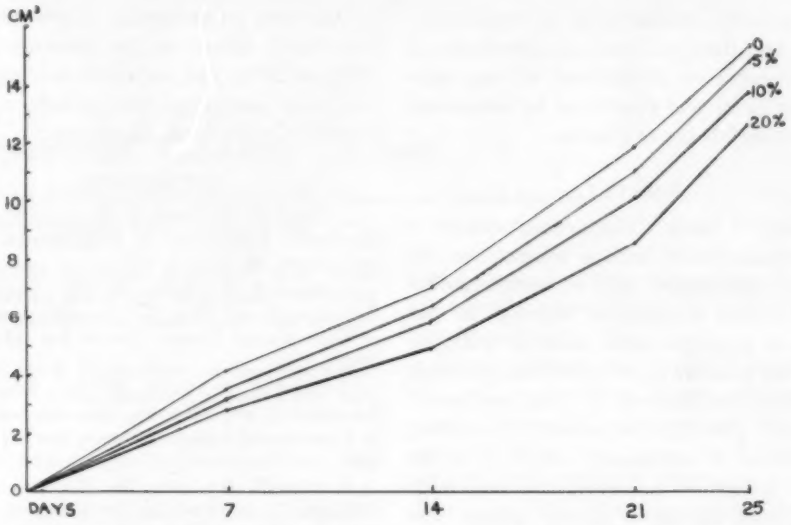

Chart 2.—Effect of galactose on Crocker rat carcinoma. All rats were on an exclusive meat isocaloric diet. Group I, control, 30 rats. Group II, 20 rats, 5% of galactose; Group III, 20 rats, 10% of galactose; Group IV, 20 rats, 20% of galactose. Average tumor size is shown in cubic centimeters.

control experiments with insulin have indicated, moderate hypoglycemia, with blood glucose concentrations of 40 to 45 mg/100 cc., had very slight, if any, inhibitory effect on Crocker rat carcinoma.

GALACTOSE

In this series of experiments, 90 rats were divided into four groups. Group I, control, 30 rats, was kept on an exclusive meat diet. Group II, III, and IV, of 20 rats each, were placed on a meat diet containing 5%, 10%, and 20% of galactose, respectively. The average rat weight was 210 gm. These diets were well tolerated by normal healthy rats, and no ill-effects were observed. No loss of weight was noted, except in Group IV.

Chart 2 summarizes the results of these observations.

The figures of Chart 2 seem to indicate that galactose does not exert, in any significant degree, antitumor activity. There was a slight inhibition of tumor growth in Groups III and IV, but this was not sufficiently large for any importance to be attributed to it. Moreover, the span of life in all groups, control as well as treated, was almost the same: Group I (control), 26 days average; Group II (5% of galactose), 29 days; Group III (10% of galactose), 31 days, and Group IV (20% of galactose), 24 days.

Galactose affected the blood glucose concentrations markedly but in a different manner than 2 DG. Table 2 gives the average figures of glucose concentrations in the four groups of rats.

Oral administration of galactose gave a rise in blood glucose concentration in rats. In four to five hours the glucose level in blood returned to normal, or almost normal, figures.

Our experiments show that while 2-desoxy-D-glucose manifests a definite anti-

TABLE 2.—*Effect of* D-*Galactose on Blood Glucose Concentrations* *

	Blood Glucose Concentrations, Mg./100 Cc., Av.			
	9 A. M.	1 P. M.	4 P. M.	8 P. M.
Group I, control	72	129	110	79
Group II, 5% galactose	77	168	122	74
Group III, 10% galactose	83	170	132	86
Group IV, 20% galactose	82	188	136	85

* Rats were kept on an exclusive meat isocaloric diet. Feed was given at about 10 a. m. and was consumed in 30-45 minutes. The blood samples were taken at 9 a. m. and at 1, 4, and 8 p. m.

tumor activity, indicative of its competitive nature, and thus is a true antimetabolite of glucose, galactose is deprived of any antitumor property and should not be considered as an antimetabolite of glucose.

COMMENT

Woolley [12] made a distinction between a true antimetabolite, with a competitive nature well manifested, and an antimetabolite with a limited antagonistic activity. In our studies on D-glucoascorbic acid, an analogue of ascorbic acid, we have shown that although the competitive activity of this compound was only partially evidenced it exerted, nevertheless, an antitumor activity.‡ In the case of galactose, however, the antitumor activity was absent, or almost absent. On the other hand, galactose possibly competes with glucose in fat metabolism, according to the work of Richter,[13] Nieft and Deuel,[14] and others.

The findings of Horowitz and King,[16] that glucose is a precursor of ascorbic acid, with the synthesis proceeding by way of glucurolactone, and probably participates in the formation of DNA, emphasize the potential importance of glucose antimetabolites in the chemotherapy of cancer. Goldstein and co-workers [15] and Sokoloff and co-workers [4] found that ascorbic acid takes part in the formation of DNA in malignant tissue. Thus, possibly, both glucose and ascorbic acid are involved in the process of formation of the glycosidic ring of DNA. Whether 2-desoxy-D-glucose, an antimetabolite of glucose, exerts its antitumor activity through the formation of DNA or by inhibiting the glycolysis of malignant cells, or both, is still unanswered. The work with the labeled 2 DG, which is now under way, may enlighten us in this respect.

SUMMARY

2-Desoxy-D-glucose, an antimetabolite of glucose, added to a meat ration in the amounts of 2%, 5%, or 10%, inhibited the growth of transplanted Crocker rat carcinoma.

Galactose, an antagonist of glucose, added to a meat ration, in the amounts of 5%, 10%, or 20%, did not exert any significant inhibitory action on the growth of transplanted Crocker rat carcinoma.

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Abnormal Elastic Fibers

Appearance in Cutaneous Carcinoma, Irradiation Injuries, and Arterial and Other Degenerative Connective Tissue Lesions in Man

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Increasing interest in the fibers and ground substance of connective tissues has been notable during the last decade. Much of the literature relating to this field has recently been reviewed and summarized by McManus,[1] Robb-Smith,[2] and Asboe-Hansen.[3] With the realization that alterations in connective-tissue constituents (other than cells) are almost invariable in association with degeneratory lesions, many histochemical tests have been devised for demonstrating changes in the ground substance and even in the collagen fibers themselves. As long ago as 1896, Unna [4] had described differences in the staining reactions of collagen and of elastic fibers in the connective tissue of the skin in various diseases. He mentioned particularly two types of fibers, namely, collagen and elastin, and also degeneratory products of these which he claimed to be distinguishable, on the basis of tinctorial reactions. In so doing, Unna brought to some finality the work of earlier investigators, more particularly the most careful studies of Neumann,[5] Schmidt,[6] and Reizenstein.[7] Since Unna's time, however, very little advance has been made in this particular aspect of the study of connective tissues and, in fact, the contributions of Unna, and of others before him, have received only superficial attention in the modern literature on this subject.

Despite present-day intense interest in the chemical components of the ground substance, in various enzymes and in the collagen fibers of the connective tissues, very little attention has been devoted to the elastic fibers. In 1939, Hass [8] reviewed extensively modern knowledge of elastic tissue, but, despite the thoroughness of these surveys, very little emerged from them of value in redirecting research into the origin of elastic fibers or of their relations to other connective tissue components. More recently, however, the isolation by Bàlo and Banga [9] of a new enzyme, "elastase," and the description by them of some of its properties [10] has led to a reawakening of interest in the elastic components of the connective tissues in various organs, in the chemical and other characters of elastic fibers generally,* and in the possible relations between collagen and elastic fibers.[13]

A so-called "increase in elastic tissue" in various organs and tissues in many different conditions has been described by a number of investigators (see Hass for literature). We became interested in this subject as part of an analysis of chronic ultraviolet irradiation dermatitis and roentgen injuries of the skin, with their frequently associated epidermal neoplasms. One of the tissue changes commonly described in the dermis in ultraviolet and other forms of irradiation damage is "basophilic degeneration" of the collagen, detectable if hematoxylin is used in the staining, and an increase in the dermal "elastic" fibers if elastic stains are used. Careful histological

From Brenthurst-Schlesinger Research Unit, Brenthurst Clinic, Johannesburg, and the Department of Physiology, Faculty of Medicine, University of Natal, Durban.

* References 11 and 12.

study revealed that the areas of "basophilic degeneration" and of so-called "elastic tissue increases" in the dermis were apparently identically located. Furthermore, these changes in the dermal connective tissues seemed to correspond with what Unna had described as collastin, collacin, and elacin. Application of the stains recommended by Unna for identifying these unusual derivatives of presently recognized connective-tissue fibers confirmed this view. However, more detailed histological analysis revealed that the fibers in the zones of "elastic tissue increase" were morphologically different from "normal" elastic fibers, even though they stained with all the accepted and generally used elastic fiber stains. We found, too, that the abnormal fibers, which stain so intensely with elastic stains, although manifesting many other tinctorial reactions known to be given by elastic fibers, to our surprise also stained with many dyes or dye-mixtures which did not stain "accepted" elastic fibers.[14]

As a consequence of these findings, we conducted a systematic survey of the staining reactions of recognized elastic fibers in arteries, veins, and other vessels and in many other tissues, including the skin, in health and in disease.

The object of this communication is to report more fully than previously [14] and to discuss briefly the possible significance of some of our findings. It is hoped that our findings will redirect attention to these changes in connective tissues which have hitherto been grouped rather vaguely and superficially under the titles "increases in elastic tissue," "basophilic degeneration of collagen," or "alterations in elastic fibers" and will at least promote further efforts to define the relationships between ground substance and the various fibers in connective tissues, more particularly elastic fibers.

MATERIAL AND METHODS

The material used in this study may be classified broadly into two categories: 1. Vascular. This included a large number of aortae and carotid arteries and many other small arteries observable in tissues such as the thymus, liver, gall bladder, kidney, etc. These vessels, and the other tissues mentioned above, were obtained either from healthy subjects who had died from acute trauma or from persons with known diseases. 2. Skin. We have devoted particular attention to the normal skin from different fields and to alterations in the connective tissue of the dermis in chronic ultraviolet irradiation dermatitis and in roentgen-irradiation lesions, old-burn scars, and other types of scars derived from cases presenting themselves at a plastic surgery clinic for cosmetic treatment or for the treatment of cancerous or precancerous lesions of the skin resulting from the above-mentioned irradiations. However, we shall confine our remarks to those aspects of elastic fibers in normal and abnormal skin which are relevant.

Histological Techniques.—Most of the sections studied were from formalin-fixed tissues, dehydrated and embedded in wax. Frozen sections stained with Scharlach R and counterstained with hematoxylin were also studied, when deemed necessary. The stains and histochemical tests applied are presented in the Table. Serial sections were used for comparing the findings in the same tissue prepared with different staining and histochemical procedures. In addition, we particularly examined the alterations in staining reactions produced in the above-mentioned tissues following digestion with elastase and trypsin enzymes. The former enzyme was initially made available to us by Dr. I. Banga and the method used was that detailed by him in a personal communication. However, we do not intend to report here on our findings following digestion with the above-mentioned and other enzymes or on the effects of these enzymes on the staining with many of the methods mentioned in the Table.

OBSERVATIONS

In view of the fact that arteries are commonly employed for the demonstration of elastic fibers, we will commence our descriptions with our findings in normal and in diseased blood vessels.

Two types of fibers were distinguishable in apparently normal medium-sized blood vessels, such as the carotid artery, following staining with recognized elastic fiber stains, viz., Weigert's, Verhoeff's aldehyde-fuchsin, and the Taenzer-Unna-Orcein methods. The two types of elastic fibers to which we refer are, firstly, fine, delicate, relatively straight fibers and, secondly, the rather coarse elastic "membranes" characteristic of large and medium-sized blood vessels. While both these types of fibers stained with the above-

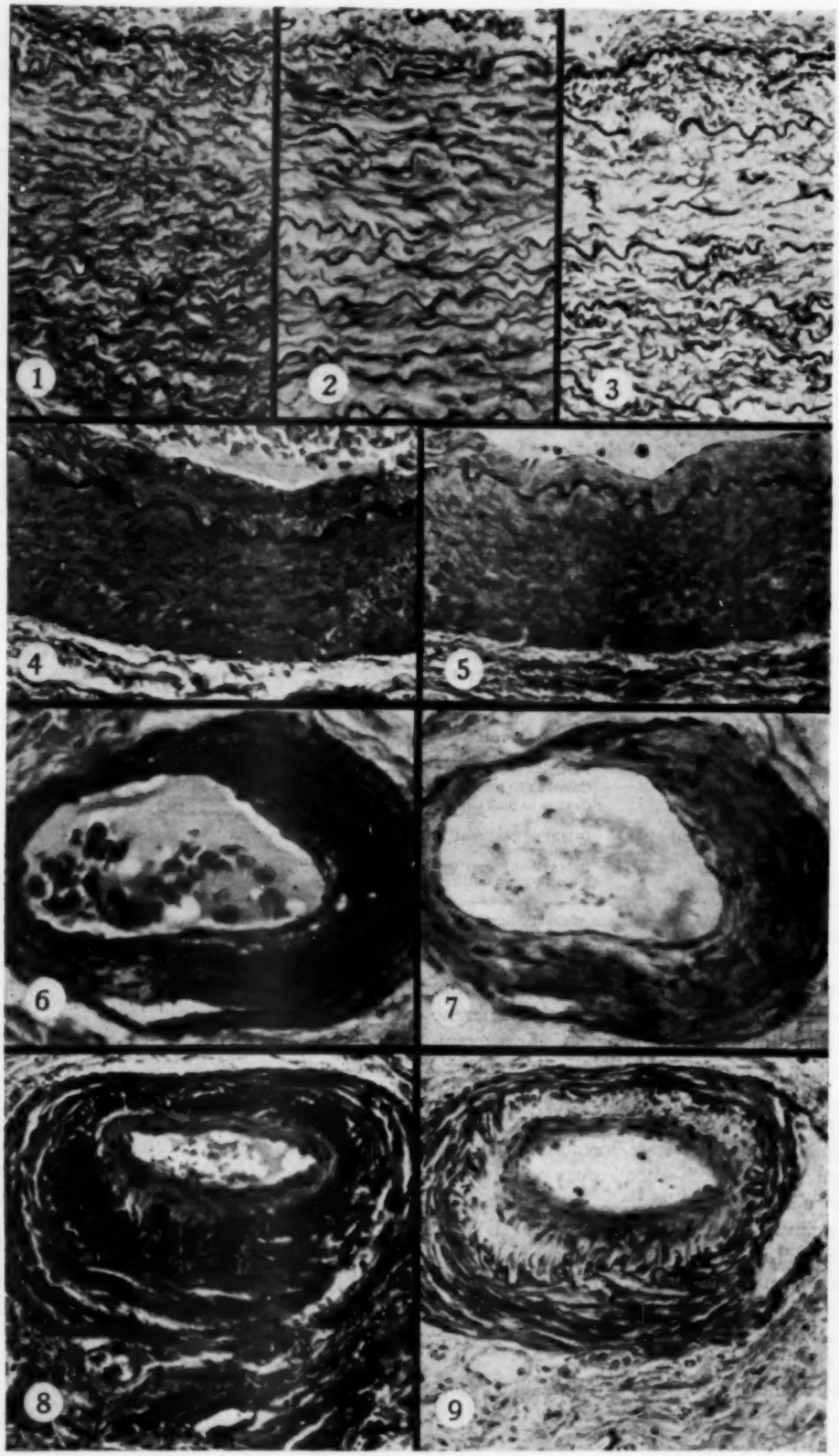
mentioned stains, most of the finer and all of the coarser fibers stain with Verhoeff's stain. Only the coarse membranes are dyed when the Mallory phosphotungstic acid hematoxylin (Mallory's P. A. H.), dilute toluidine blue, or the Rinehart-Abul Haj methods are applied (compare Figs. 1, 2, and 3). With Weigert's, Verhoeff's or Heidenhain's iron hematoxylin methods the so-called elastic membranes are shown to consist of an inner paler-staining "core" and a surrounding deep blue or blue-black "coat." The latter, i. e., outer "coat" stains selectively with Mallory's P. A. H. and iron hematoxylin, while the entire membrane stains diffusely with orcein. Critical examination of specimens stained with Mallory's P. A. H. shows occasional oval or round, "moth-eaten," clear spaces in the deep purple "coating" of the wavy membranes. These same clear, "moth-eaten" areas may be seen when a wavy membrane is cut obliquely. In specimens stained with the Wilder reticulum method, it can be shown that a fine and almost continuous reticulum fiber membrane extends along the outer borders of the wavy elastic membranes, identical with that described by Smith and co-workers.[15] From these outer reticulum "tram-line"-like reticulum fibers, there extend numerous finer reticulum fibers connecting the elastic membranes with the interstitial collagen and with reticular fibers around smooth muscle bundles in the media of blood vessels. The junction between the continuous reticulum fibers surrounding the elastic membranes and the finer interstitial reticulum fibers is usually punctuated by numerous pieces of coarse granular reticulin-positive material. When Van Gieson's stain is applied as a counterstain to Wilder preparations it can be shown that the "core" of the elastic membrane, lying between the reticulum boundaries, stains a fairly pale yellow, while the true collagen fibers between the elastic membranes and the smooth muscle bundles stain bright red.

Tinctorial Reactions of Elastic Fibers in Healthy Dermis and Arteries Compared with "Elastotically Degenerated" Fibers

Stain	"Normal" Elastic Fibers, Dermis or Arteries	"Elastotically Degenerated" Fibers, Dermis or Arteries
Orcein	Deep red-brown	Pale to deep reddish-brown
Verhoeff's	Dark blue-black	Pale to dark blue-black
Weigert's	Deep purple-blue	Deep purple-blue (+ eosinophilic "granules")
Gomori's aldehyde-fuchsin	Deep red-purple	Deep red-purple to pale red
Periodic acid-Schiff	Red borders	Irregular red "pools" and pink fiber borders
Iron hematoxylin	Gray, black, or brown	Gray to jet-black fibers, rods, and "pools"
Safranin water-blue (Unna)	Pink to red	Pink to deep red
Van Gieson	Yellow with thin red fiber outlines	Yellow with irregular red "granules"
Wilder's reticulum and basic fuchsin	Red fiber "cores" with thin black outlines	Irregular coarse granular and fine black fibers
Ponceau 2R-light green *	Bright pink-red	Irregular blotchy "gray" with red and yellow patches
Hematoxylin and eosin *	Pale pink	Pale blue-gray + pink "granules" and threads
Toluidine blue *	Colorless to pale blue	Deep blue-green coarse fibers and "granules"
Mallory's triple *	Orange to pink	Completely chromophobic with scattered orange or blue "granules"
Mallory's phosphotungstic acid hematoxylin *	Bright purple "coat" with orange "core"	Pale orange with occasional purple threads
Rinehart-Abul Haj * †	Pale yellow "cores" with bluish-green outlines	Dirty pale green with scattered blue "pools"

* Stains providing clearly distinguishable tinctorial differences between "true" elastic fibers and "elastotically degenerated" fibrillar aggregations.
† Modified Hale's method for acid mucopolysaccharides.[52]

"Fibrinoid degeneration" in vessel walls, as definable by the accepted staining reactions,[16] is easily distinguishable with the above-mentioned stains, in that we have found fibrinoid to stain rather intensely and homogeneously with iron hematoxylin, Verhoeff, Weigert, and orcein stains and a deep purple with Mallory's P. A. H. In fact, even slight localized "fibrinoid" changes are sharply depicted with orcein and Mallory's phosphotungstic acid hematoxylin (P. A. H.), so



Figures 1 to 9

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much so, that these latter stains can be strongly recommended for the precise delineation of fibrinoid in any organ. Although fibrinoid and elastic fibers both stain with orcein and P. A. H., it is possible to distinguish between these two types of material, by virtue both of the absence of fibrillar structures and of the intense purple color of the former. The homogeneous staining of the connective tissues in fibrinoid degenerations seems to be due to the merging of the staining of preexisting fibers and of the matrix in which these lie.

It is further possible, by critical analysis of morphology as well as by tinctorial reactions, to distinguish sharply between elastic fibrils, elastic membranes, and "fibrinoid degeneration," on the one hand, and what we have called "elastotically degenerated" connective tissue, on the other.

In many pathological states of blood vessels, so-called "reduplication" of the internal or external elastic laminae is described. Also recorded are degeneratory "increases" in the elastic fibers of the media, especially in small arteries and following irradiation injuries. We have examined blood vessels from various sources for such "increases" in elastic tissue. Morphologically, in our opinion, these so-called new elastic fibrils are frequently abnormally thick, beaded, granular, or unusually coiled, after the manner fully described by Unna [4] in senile degeneratory lesions of the dermal fibers. Examples of such increases in elastic fibers are depicted in Figures 4, 6, and 8. Similar changes in vessels are the rule in the obliterative endarteritis of thromboangiitis obliterans and, especially, in chronic irradiation injuries.† Apart from their morphological peculiarities, these additional elastic fibrils and coarser fibers, while consistently demonstrable with Verhoeff's, Weigert's, and orcein stains, are not stainable with Mallory's P. A. H. The differences in staining reactions between the "true" elastic membranes of blood vessels and the so-called "new" elastic fibers are clearly demonstrated by comparing Figures 4 and 5, 6 and 7, and 8 and 9; serial sections of the same vessels, in each case, were stained with one or another recognized elastic stain (in the left-hand, even-numbered sections in each case) and with Mallory's P. A. H. (phosphotungstic acid hematoxylin) in the remaining figures. It may well be that the increase in fine intimal "elastic" fibers in the vessel depicted in Figure 4 does in fact represent a "true" increase in those fine elastic fibers which, as shown in Figures 1 and 3, and in Figures 12 and 13, are stainable only with recognized elastic fiber stains. However, in the cases of the medial changes shown in Figures 6 and 8 the material stainable with elastic stains is coarsely granular, for the most part. Furthermore, the morphology of the media is distorted in each case, as

† References 8, 17, and 18.

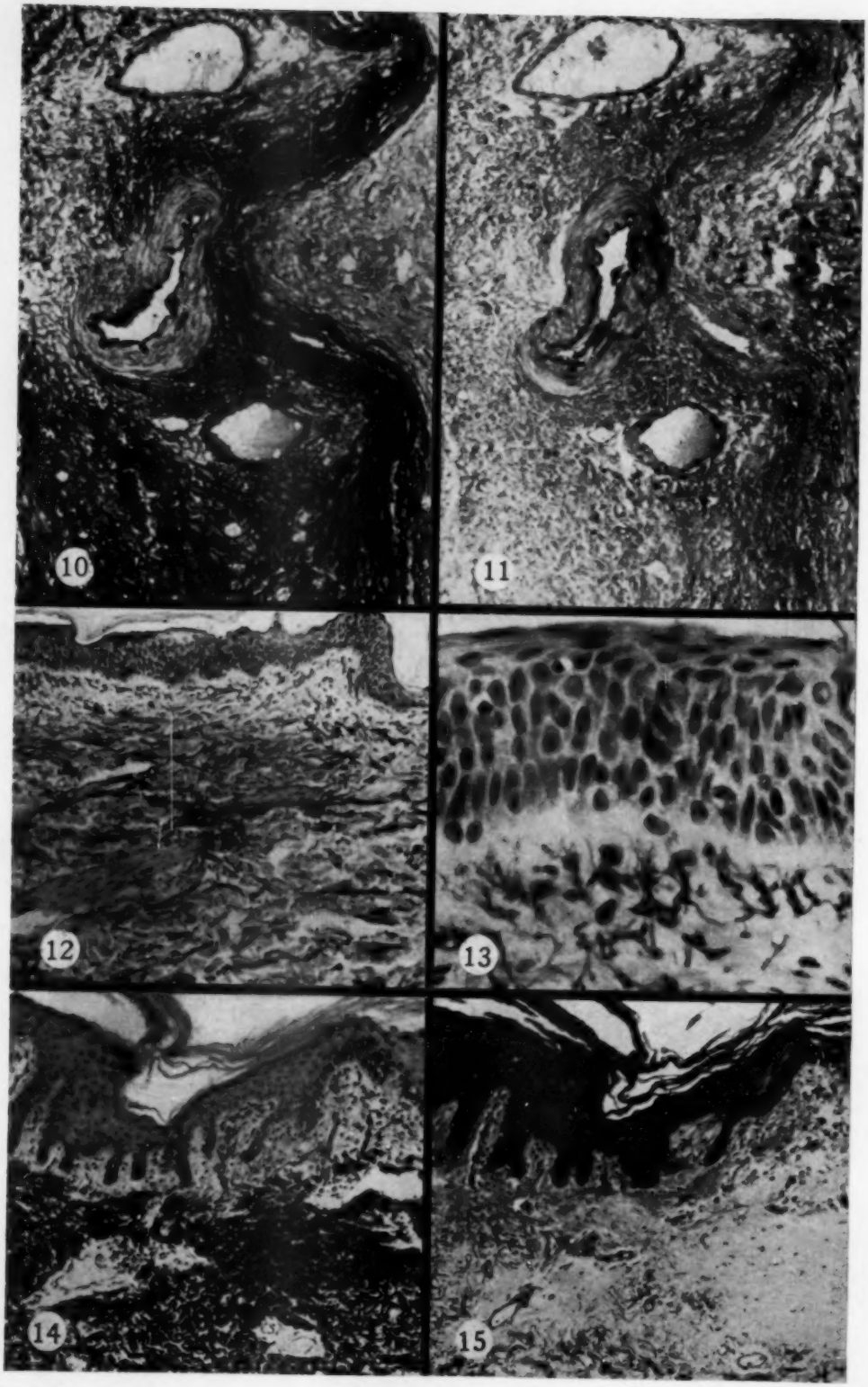
EXPLANATION OF FIGURES 1 TO 9

Figs. 1, 2, and 3.—Serial sections of the same normal carotid artery stained with Weigert's elastic method, Mallory's phosphotungstic acid hematoxylin (P. A. H.), and orcein, respectively. Note that in Figures 1 and 3 coarse elastic membranes and fine elastic fibers are stained, whereas in Figure 2 only the coarse membranes are stained; all, × 200.

Figs. 4 and 5.—Serial sections of a mesenteric vessel stained with Weigert's stain (Fig. 4) and with Mallory's P. A. H. (Fig. 5). In both figures the well-defined internal elastic lamina are shown. The fine, reduplicated elastic fibers in the intima, clearly stained with Weigert's stain, are not stained in Figure 5; both, × 200.

Figs. 6 and 7.—Serial sections of another mesenteric vessel, showing an eccentric medial thickening containing coarse granular Weigert-positive material (Fig. 6) which is unstained by Mallory's P. A. H. (Fig. 7), the latter stain, however, demonstrating a marked local increase in medial smooth muscle; both, × 440.

Figs. 8 and 9.—Serial sections of an abnormal blood vessel in the tunica propria of a diseased gall bladder stained with Weigert's stain (Fig. 8) and with Mallory's P. A. H. (Fig. 9). In the former the medial coat can be seen to be constituted very largely of coarsely granular Weigert-positive material which has failed to stain with Mallory's P. A. H. However, in Figure 9 the remains of the wavy internal elastic membrane can be clearly seen (especially between 4 and 8 o'clock on the vessel); both, × 200.



Figures 10 to 15

(See legends on opposite page)

shown by the unilateral thickening of this layer. The intimal muscle also seems unduly developed in the vessel shown in Figure 8. That there seems to be a degeneratory change in the media of this latter vessel was substantiated by the appearance of a specimen stained for reticulin (Wilder's method), which demonstrated a marked increase in fine *Gitter Fasern*. The apparent increase in thick elastic membranes in this vessel (Fig. 8) seems to be attributable to an alteration in the reticulum enmeshing the smooth muscle fibers of the media, or perhaps even of the intima. That this interpretation seems correct is borne out by the appearance of the same specimen stained with Mallory's P. A. H. (Fig. 9), in which the fragmented but still distinguishable remains of the wavy elastic lamina can be distinguished clearly. Staining of such blood vessels with Van Gieson's connective tissue method also revealed an undue increase in collagen, a finding which was beautifully demonstrated in other specimens of the same vessel stained by the Rinehart-Abul Haj modification of Hale's technique for mucopolysaccharides.

These changes in blood vessels seem to be due to an accumulation of fibrillar material in the wall of the vessels which, while stainable with recognized elastic fiber stains, nevertheless fails to stain with some dyes which may color elastic fibers, e. g., Rinehart-Abul Haj method, which colors elastic membranes yellow, and Mallory's P. A. H., which colors the same membranes deep blue. These coarse, granular fibrils also stain with other dyes which do not color "true" elastic membranes or fibrils (Table). Such changes in the staining reaction of the various layers of the blood-vessels with elastic, collagen, and reticulum stains are invariably found as precursors of the deposition of "true" collagen in degeneratory medial changes in vessels of all sizes when so afflicted. In particular, the accumulation of elastotically degenerated fibers in blood vessels seems to be a frequent precursor of calcification in these loci. All these observations taken together seem to indicate that these abnormal fibers may represent some alteration in the collagen or reticulin fibers, or perhaps even an abnormal product formed during the active deposition of new collagen following an injury to the area. We have therefore called these lesions "elastotically degenerated fibers."

Further evidence of the probable degeneratory nature of these elastotically degenerated fibers is available from two other sources—namely, from the gall bladder and from the skin. Riopelle [19] reported an apparent increase in elastic fibers in the diseased gall bladder. Closer examination of his published figures, in the light of our previous studies, led us to suspect that Riopelle had, in fact, described elastotic degeneration of the gall-bladder wall. At our request, Dr. Riopelle sent us several unstained serial

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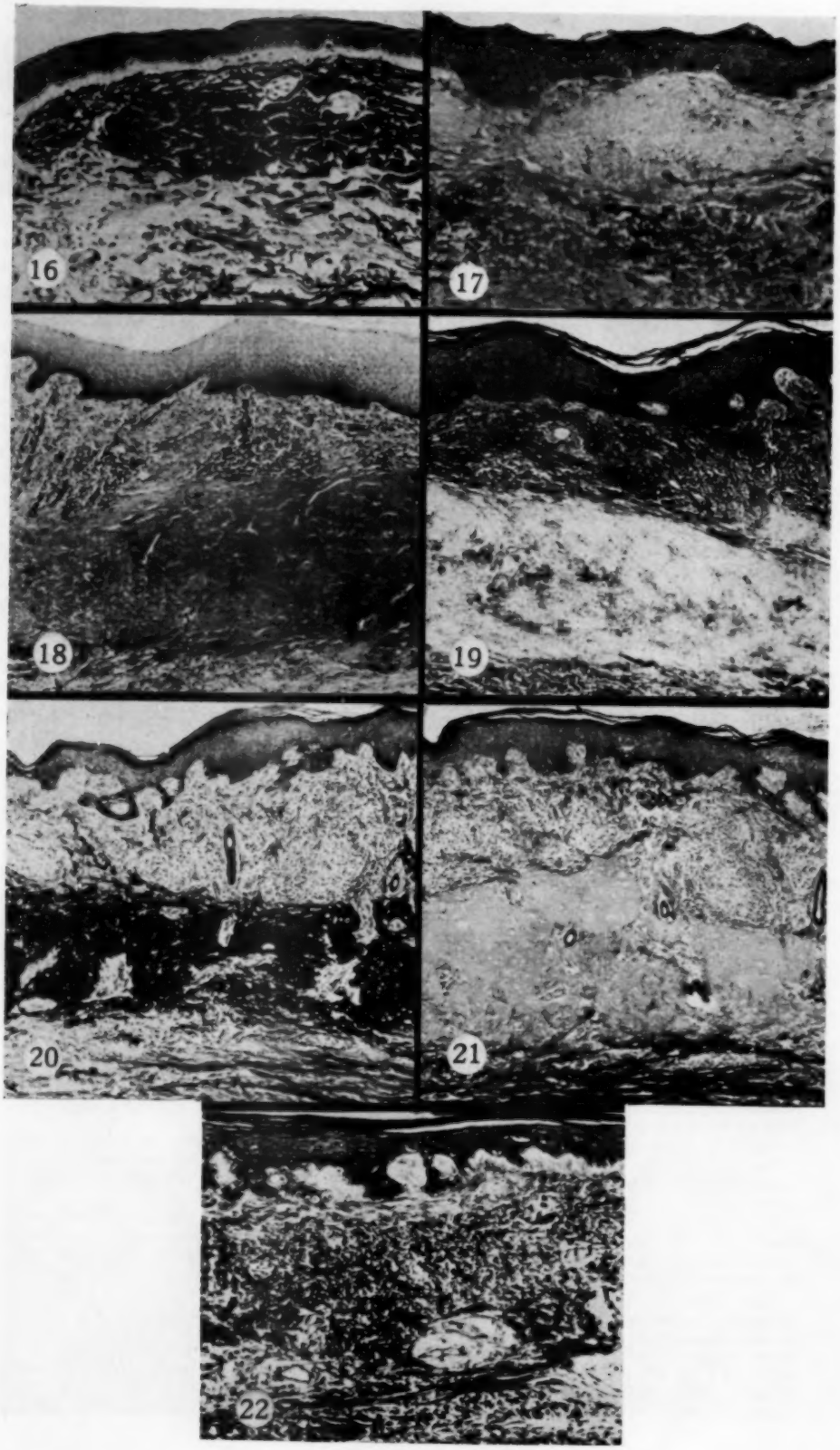
EXPLANATION OF FIGURES 10 TO 15

Figs. 10 and 11.—Serial sections of the tunica propria of an abnormal gall bladder, showing in Figure 10 a broad zone of darkly staining orcein-positive fibers and a clearly depicted internal elastic lamina in the central vessel. In Figure 11, the Mallory P. A. H. sharply stains the internal elastic membrane of the central vessel but not the surrounding mass of fibers which are so clearly shown with orcein in Figure 10; both, × 100.

Fig. 12.—Normal skin of anteromedial aspect of the arm stained with orcein and showing the coarse orcein-positive elastic "membranes" distributed between the collagen bundles in the stratum reticularis of the dermis. The fine subepidermal elastic network is just distinguishable at this power; × 100.

Fig. 13.—High-power view of orcein-stained section of skin shown in Figure 12. Here the denditric-like, fine elastic fibers are clearly shown immediately below the epidermis. These fine fibers do not stain with Mallory's P. A. H. but are demonstrable with Weigert's, Verhoeff's and the Taenzer-orcein methods for elastic fibrils; × 440.

Figs. 14 and 15.—Serial sections of the skin of the dorsum of the hand of a patient with chronic ultraviolet irradiation dermatitis with numerous carcinomata in other sections. The acanthosis, the alterations in the stratum papillaris of the dermis, and the thick band of "excessive" elastic tissue (appearing black in Fig. 14, orcein-stained, but unstained and therefore gray in Fig. 15, Mallory's P. A. H.) are all clearly shown. Note the coarse very wavy character of the so-called excessive elastic fibers in Figure 14, which are unstained in Figure 15; both, × 100.



Figures 16 to 22

(See legends on opposite page)

sections of some of his specimens. These we subjected to our battery of stains. One of the results of our investigation is apparent from an examination of Figures 10 and 11, presented here for comparison with the preceding figures. Figure 10 shows the staining reactions to orcein in one of Dr. Riopelle's specimens. Here it can be seen that the internal elastic lamina of the vessel, lying centrally in the figure, is sharply delineated. In addition, masses of unduly wavy and thick fibers, in the tissue around the vessel, are also shown to be heavily stained by this method. When a neighboring section of the same specimen was stained with Mallory's P. A. H., the internal elastic membrane of the centrally located blood vessel was again clearly definable as a blue-black wavy line. However, only a few fibers in the surrounding tissue were stained purple or blue, most of them being stained a pale yellow (not the deep orange of normal collagen or the diffuse purple of fibrinoid when stained with Mallory's P. A. H.). Such Mallory-P. A. H.-negative fibers also fail to stain with recognized collagen stains such as Van Gieson or the aniline blue in Mallory's triple connective tissue stain. We therefore conclude that these fibers in the perivascular tissue of the tunica propria in this gall bladder are neither collagen nor true elastic fibers. On the tinctorial criteria here suggested, they may be regarded as elastotically degenerated fibers.

Our findings in the skin are of equal if not greater interest for understanding the conditions under which elastotic degeneration of fibers arises. Particular interest relates to the study of elastotic degeneration in the skin for the following reasons: Firstly, elastotic degeneration supervenes as a normal process of ageing, especially in facial skin; secondly, this change in connective tissue fibers of the dermis can be induced, in man and in animals, by a variety of physical and chemical methods; thirdly, we have evidence, from experiments in human subjects (outlined below), suggesting that this change in the dermis represents some alteration in the metabolism of the connective tissues and, in certain circumstances, may be associated with (if not responsible for) epidermal hyperplasia and invasiveness.

The arrangement of elastic fibers in the normal skin at different ages has received considerable attention by Pasqualino [20] and by Cowdry.[21] We do not intend detailing here the differences in the elastic fibers and of collagen in different skin fields, and in any single skin field at different ages. This subject merits separate attention. In outline,

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EXPLANATION OF FIGURES 16 TO 22

Figs. 16 and 17.—Serial sections of a scar on the back following a severe x-radiation burn, stained with orcein (Fig. 16) and with Mallory's P. A. H. (Fig. 17). Below the scar-like epithelium can be seen a broad zone of orcein-positive granular, fibrillar material usually referred to as "excess elastic fibers," which are unstained by Mallory's P. A. H.; both, × 100.

Figs. 18 and 19.—Serial sections of skin from the back of the hand of a patient with carcinoma treated with x-rays, stained with dilute toluidine blue (Fig. 18) and with Mallory's P. A. H. (Fig. 19). Note that in Figure 18 the "elastotically degenerated" zone stains diffusely but heavily, whereas this zone is unstained in Figure 19. Note the well-defined highly cellular, altered subepidermal stratum papillaris with numerous darkly stained (metachromatic) mast cells in Figure 18; both, × 45.

Figs. 20 and 21.—Serial sections of the skin of the dorsum of the forearm from another case of chronic ultraviolet irradiation dermatitis, associated with neoplasms, showing the hyperkeratotic and acanthotic epidermis, the highly cellular abnormal subepithelial layer devoid of elastic fibrils but with a little collagen (Fig. 21, Mallory's triple stain), and the broad band of fibers heavily stained with Weigert's stain (Fig. 20) but unstained (chromophobic) with aniline blue in Figure 21. The deep, dermal collagen fibers, below the chromophobic elastotically degenerated band, stain heavily with Mallory's connective-tissue stain in Figure 21; both, × 45.

Fig. 22.—Skin abutting an old burn scar, in the temporal region of the head, which had undergone malignant transformation. In this specimen some areas showed a zone of coarse wavy fibrils which stained blue-black with Mallory's P. A. H. as well as with Weigert's stain. In neighboring regions, this same connective tissue zone was Weigert-, Verhoeff-, and orcein-positive but was negative with Mallory's P. A. H. stain; × 100.

however, it can be stated that, with the usual elastic fiber stains, at least two generally recognized types of elastic fibers can be distinguished in the dermis, namely: (1) coarse, membrane-like structures, lying between the thick collagen bundles in the stratum reticularis of the dermis (Fig. 12), and (2) very fine dendritic-like fibers ramifying immediately subepidermally in the stratum papillaris (Fig. 13) and in the equivalents thereof surrounding hair follicles and sebaceous glands. The processes of the fine subepidermal elastic network interdigitate with delicate collagen and reticulin fibers characteristic of the stratum papillaris. In aged facial skin, in particular, and following chronic ultraviolet, x-ray, or radium injuries to the dermis, the description of the normal organization of the cutis may be distinguished by at least three important criteria: Firstly, the entire dermis becomes thicker as a rule; secondly, the stratum papillaris may become replaced by a thick layer of tissue filled with round cells, young fibroblasts, and numerous mast cells, rich in reticulum fibers and relatively poor in collagen (Figs. 14, 18, 20, and 21). In this cellular subepidermal layer coarse fragments or granules of apparently degenerated elastic fibers may be present, but the fine subepidermal network depicted in Figure 13 disappears. Thirdly, a broad band of tissue appears between the modified stratum papillaris and the residual stratum reticularis of the dermis. This layer is constituted of highly coiled fiber bundles through which the hair follicles, sebaceous and sweat glands, and blood vessels course. As these structures penetrate this broad band, which stains heavily with the elastic stains, they are invariably surrounded by a layer, varying in width, of relatively normal stratum-papillaris-like connective tissue (Figs. 14, 16, 20, and 22).

With certain stains, this band, below the modified stratum papillaris, may seem to be virtually devoid of fibers and therefore rather homogeneous (Figs. 15, 18, and 21). The thick, highly coiled and/or wavy fibers which constitute this band were originally described by Neumann [5] and were subsequently characterized more fully by Schmidt,[6] Reizenstein,[7] Unna,[4] and Pasqualino [20] and, more recently still, by Montgomery,[18] Vernoni,[22] and Lever.[23] All these authors agree that these fibers are regularly encountered, as clumps or as broad interrupted bands, in senile skin and, especially, in skin injured by chronic ultraviolet, roentgen, or radium irradiation. Using different staining reactions, Unna described fibers in such injured or aged skin and classified them into elastin (normal elastic fibers), elacin (basophilic elastin), collacin (degenerated collagen retaining the form of collagen but staining like elacin), and, finally, collastin (a combination of collagen and elastic staining in one "granule"). In relatively recent years, Montgomery [18] has described these fibers in less detail than did Unna, and he refers to them broadly as "homogenized and hyaline-like" or he states that

> Basophilic staining of collagen fibres and merging of collagen and elastic tissue may result in the two types being indistinguishable one from the other. The staining of elastic tissue fibres with ordinary hematoxylin-eosin preparations is a phenomenon frequently encountered in association with all degrees of radiodermatitis involving the exposed surfaces.

Vernoni,[22] on the other hand, refers to various morphological alterations in the dermal tissues, such as "convoluted hypertrophic elastic fibers" with "amorphous masses resulting from the blending of the hypertrophic elastic fibers" or to "degenerating elastic fibres." Vernoni also mentions "elastic material showing granular degeneration."

We have been able to confirm all the aforementioned meticulous morphological descriptions and tinctorial reactions detailed by workers in the latter half of the last century. However, we have now been able to add further tinctorial criteria for characterizing these fibers (Table). In particular, we wish to stress that this band of abnormal connective tissue fibers, which lies between the altered stratum papillaris above and the deeper portions of the stratum reticularis of the dermis below, fails to stain with accepted collagen stains (Van Gieson's, the aniline blue of Mallory's triple connective tissue

stain, e. g., Fig. 21, and Mallory's phosphotungstic acid hematoxylin, Figs. 15, 17, and 19); they stain with all stains used to demonstrate elastic membranes and fibrillae and, for the most part, fail to stain with many dyes which stain elastic membranes sharply but which do not usually stain more delicate elastic fibrillae (*see above*). In addition, these abnormal fibers stain yellow with the picric acid in Van Gieson's mixture and contain very large numbers of coarse and fine argyrophil reticulin fibrils. Metachromatic and periodic-acid-Schiff-positive "pools," seemingly derived from disorganized homogenized fibers, are also commonly found in these zones. Mast cells are usually numerous within these areas, but even more so in the overlying modified unduly cellular stratum papillaris and in the perivascular and periductal residual relatively "normal" connective tissues. These fiber masses also stain sharply blue-green with dilute thionine or toluidine blue (Fig. 18), dyes which barely color normal collagen and which stain arterial elastic membranes pale sky-blue. These degenerated fiber masses in the dermis are apparently rich in mucopolysaccharides and possibly also in other carbohydrates, as shown by the positive reactions with dilute toluidine blue or thionine, with periodic acid-Schiff stain, and with the modified Hale's method devised by Rinehart and Abul-Haj.

Routinely, we distinguished these abnormal fiber masses from normal collagen or elastic fibers by comparing the findings (following the application in serial or neighboring sections) of one or more of the recognized elastic fiber stains with the reactions encountered in specimens stained with Van Gieson's, Mallory's triple, and, especially, Mallory's P. A. H. The chromophilia of this band of altered fibers with elastic stains (Figs. 14, 16, 18, and 20) and its chromophobia with either Mallory's triple or P. A. H. stains (Figs. 15, 17, 19, and 21) are clearly evident by comparing serial sections of the same specimens (presented here in pairs as Figs. 14 and 15, 16 and 17, and 20 and 21). The differences in staining reactions with toluidine blue and with Mallory's triple stain, in another specimen, are shown in Figures 18 and 19. A full appreciation of the differences in the tinctorial reactions can only be achieved when colors (and not just shades of black and gray) are used for representing these distinctions. However, expense of color reproduction unfortunately prevents this highly desirable presentation of our findings.

The staining reactions of normal collagen, elastic membranes, and "fibrinoid degeneration" with Mallory's P. A. H. are especially interesting. This stain, which is only a modified hematin, and which is apparently not used nearly as widely by pathologists as it merits, has many features to recommend it highly to pathologists or cytologists interested in connective tissues, neuroglia, muscle structure, epithelia in general, and the epidermis and its derivatives in particular. Normal collagen fibers stain a delicate orange, while epidermal tonofibrils, keratin, muscle fibers and striations, elastic membranes of blood vessels, fibrin in exudates and hemorrhages, and fibrinoid stain various shades of blue or deep purple. The delicate subepidermal elastic fibrils, so sharply delineated with Weigert's method and especially with orcein (Figs. 12 and 13), are not demonstrable at all by Mallory's P. A. H., whereas coarse elastic membranes in vessels and in the corium stain a striking blue-black (Figs. 2, 10, and 17). The abnormal pseudoelastic fiber masses, in blood vessels, gall bladder, dermis, and elsewhere described above, for the most part, are almost chromophobic and appear as a pale yellowish virtually homogeneous mass. Within the latter chromophobic masses may be found coiled orange-staining collagen fibers which may be stained purple along the edges or in striae but not blue-black as are the elastic membranes of arteries and the cutis.

We have shown, by experiments with human and animal dermis, that mechanical or other trauma to dermal collagen results in an immediate alteration of the staining of collagen fibers from orange to deep purple. In such traumatized collagen it is easy to

pick out single fibers which along their length change color quite sharply from orange to purple. These fibers correspond with what has been described in the literature as "fibrinoid degeneration." We know of no better method for demonstrating such "fibrinoid" change than Mallory's P. A. H. There is a distinct difference in the staining reactions of fibrinoid elastic membranes, the former staining bright purple; the latter, blue to blue-black. One can often find purple fibrinoid, degenerated fibers extending from the deep dermis into the elastotically degenerated masses, where they seem to disintegrate into coarse, then into fine, colloid-like granules which apparently undergo further chemical change, ultimately becoming chromophobic to Mallory's P. A. H. Such phenomena are easily demonstrable in old scars resulting from thermal or acute irradiation burns, which are months or years old. Such a specimen is shown in Figure 22, where clumps of coiled purple fibers can be seen in abundance in the exact location where elastotically degenerated fiber masses (normally chromophobic to Mallory's P. A. H.) are found elsewhere in the same specimen.

Thus, with Mallory's P. A. H. the following fiber structures can, with a little experience, be distinguished: normal collagen (orange), fibrinoid degeneration (purple), chromophobic pseudoelastic or elastotically degenerated fibers (very pale yellow to chromophobic), and stages between the above. We believe that these staining reactions with Mallory's P. A. H. of dermal and other fibers provide an excellent basis for determining the mode of origin of elastotically degenerated fibers. We believe that the exact chemical basis for these profound differences in fiber staining with hematin (and with the other stains mentioned above in the Table) deserves careful attention. Mallory's P. A. H. may prove particularly useful as a means of determining the mode of origin of "true" elastic membranes, fibrinoid degeneration of collagen, and elastotically degenerated fibers in the connective tissues, more especially if it is used in conjunction with known histochemical methods and with enzymes which attack the ground substance or the fibers of connective tissues.

COMMENT

In our view, Unna and his contemporaries and, in the present century, Bierich [24] and Bierich and Rosenbohm [25] were among the first modern students of pathology to direct attention specifically to diseases of collagen. Interest in changes in the noncellular components of the connective tissues has increased considerably, following the coining of the term "collagen diseases" by Klemperer and his associates (see References 1, 2, and 3 for literature) and the recruitment into pathology of biochemists, physicists, and histochemists who have devoted attention to the characterization of morphologically distinguishable changes in connective tissues. Despite the elucidation, firstly, of the submicroscopic morphology of connective tissue fibers ‡ and, secondly, of their chemistry as revealed by their solubility or digestibility by recently discovered enzymes,§ the changes in dermal elastic fibers, carefully described by earlier workers, have received little attention.

While collagen and reticulin fibers and, especially, the connective tissue matrix or ground substance have received rather close attention during the last decade, the origin, chemistry, and pathology of elastic fibers have only recently been the subject of interest.[8] As stated by Hass and, subsequently, by Robb-Smith,[2] "Elastica formation is an enigma." Until recently, the meticulous descriptions of changes in dermal collagen and elastin by Unna and his contemporaries either have been overlooked or have been mentioned merely for completeness of bibliographic citation. Investigators who have in recent years described their microscopic findings concerning alterations in connective tissue fibers and, especially, in elastic fibers, in a variety of dermatological conditions ‖ and in various vascular degenerations,¶ have

‡ References 26 and 27.
§ References 9-11, 28, and 29.
‖ References 8, 22, and 30-36.
¶ References 15 and 37-41.

not clarified the nature of these fibrillar changes. In fact, there is hardly a single modern description of the histology of human and of experimentally induced skin cancers which does not refer simply to "an increase in the elastic tissue of the dermis." Similar citations could be made for the descriptions of the early stages of vascular degeneration associated with a so-called increase in elastic fibers in blood vessels.

Part of our own research program is devoted to clarifying various aspects of skin carcinoma, occurring spontaneously in man and induced experimentally in animals. Careful study of the dermal accumulations of the morphologically unusual fibrillar material, described above and generally dubbed "an increase in elastic fibers," led us to suspect that these accumulations of unusual fibers were not, in fact, unmodified elastic fibers. It is apparent, from the above descriptions, that these unusual clumps of dermal fibers, as well as certain fibers which may appear in vessel walls and in the gall bladder and other tissues during degenerative diseases, stain with some dyes (e. g., Weigert's, Verhoeff's, orcein, and Gomori's aldehyde fuchsin, in particular) but not with all the stains which sharply delineate apparently "normal" elastic fibers. On the other hand, these same fiber clumps may stain with several dyes or dye mixtures which do not stain "normal" elastic fibers.

The conclusion, therefore, seems justified that the accumulation of clumps of morphologically abnormal fibers, in the dermis in particular, to which reference was made above as simulating that "increase in elastic fibers" described by many modern authors, cannot be regarded as being constituted of unaltered elastic fibers, either on morphological or on tinctorial criteria. We suggest the name "elastotically degenerated fibers" for these masses, since they are morphologically distinguishable from "normal" elastic fibers, even though they usually stain with the generally used elastic stains. As indicated in the Table, they can consistently be differentiated from "normal" elastic fibers by the use of several stains.

These elastotically degenerated fibers seem to be derived primarily from alterations in the composition of preformed collagen, but they may perhaps arise as a result of disturbances in those metabolic processes which lead to the formation of normal reticular, collagen, or elastic fibers. The former possibility concerning the origin of "elastotically degenerated fibers" is perhaps supported by our recently recorded finding [42] that, following the grafting of trypsin-treated autodermal or homodermal tissue in man, numerous plaques or even extensive beds of "elastotically degenerated" tissue may appear in the dermis of the host area. These "elastotically degenerated" fiber masses may have been derived from the alteration of the original collagen of the grafted tissue. On the other hand, the grafted dermal collagen may have induced a localized change in the metabolism of the connective tissue in the host site, resulting in the deposition of new fibers which are "elastotically degenerated" *ab initio*, a possibility in conformity with the latter of the above two suggestions. Many stages in the formation of these abnormal fibers in dermis can be defined on the basis of tinctorial reactions with the aid of the above-mentioned dyes. The final product of such elastotic degeneration, particularly in the dermis, can be sharply distinguished from an increase in "true" elastic fibers.

Our findings, from the study of blood vessels and of the dermis, indicate that the elastotic degeneration of connective tissue fibers is of more than passing interest. In the case of blood vessels and of the gall bladder we have evidence to suggest that the accumulation of "pseudoelastic" fibers, or what we have named elastotically degenerated fibers, is frequently associated with or followed by calcification of the affected tissues. This evidence amplifies the observations previously recorded by Lansing and his associates,# who maintained that a close correlation exists between "ageing" of elastic fibers and calcification in blood vessels.

References 37, 38, and 43.

Our findings in blood vessels, particularly with the use of Mallory's P. A. H. stain, recognized elastic stains, dilute toluidine blue, and the Rinehart-Abul Haj stain for mucopolysaccharides, on the one hand, and Van Gieson stain, Wilder's method, and Masson's and Mallory's triple stains, on the other hand, indicate that the order of events in the degeneratory changes in blood vessels, leading ultimately to vascular hardening with or without calcification are as follows:

1. An alteration in ground substance as shown by changes in the amounts and distribution of mucopolysaccharides
2. An increase in the amount of collagen present, with or without a disturbance in the formation of collagen fibers, culminating in
3. Elastotic degeneration of the affected vascular wall
4. Calcification

From an examination of the photomicrographs published by Riopelle we suspected that what he described as an "increase in elastic fibers" in the gall bladder was, in fact, what we have defined above as elastotic degeneration of connective tissue fibers, probably of collagen. This suspicion was confirmed by examination of his material through the staining procedures detailed above. We feel similarly about the photomicrographs published by Lansing and his associates in the above-mentioned works, as examples of an increase or "progressive splitting and ageing of elastic fibers of the internal elastica." We also believe it possible that the increases and clumping of "elastic fibers" in ageing vascular homografts, described and portrayed in detail by Sauvage and Harkins,[40] would also prove to be elastotically degenerated tissue fibers. These latter suspicions remain to be confirmed. If they are, then, in accordance with our findings recorded and portrayed above (Figs. 4 to 9), many accepted descriptions of vascular degenerations may require amplification with particular attention to the occurrence of and relationship between elastotic degeneration of connective tissue and the pathogenesis of calcification and of other vascular degenerations.

We cannot overlook the possibility that the supervention of elastotic degeneration of connective tissue in vessels and, especially, in the skin may represent either a degeneration of preexisting collagen or a derangement in those normal biochemical processes leading to the formation of collagen, reticulin, and/or elastic fibers from ground substance. All our studies of different types of "scars" in the skin and in internal organs like the cirrhotic liver, fibrotic gall bladder, kidney, and blood vessels indicate that an increased deposition of "true" elastic fibers is a rare event. Earlier workers have described the development of excessive quantities of elastic fibers in "scars" such as in the fibrotic areas in cirrhotic livers. Our findings indicate that these are not increases in "true" elastic fibers but rather elastotic degeneration either of preexisting reticulin or collagen in the scar tissue or the formation of elastotically degenerated material, *de novo*, in old and avascular scars. This latter suggestion is substantiated by the fact that such elastotic degeneration is a regular concomitant of chronic irradiation lesions in the skin in which obliterative endarteritis with consequent suspected avascularity is common if not invariable. However, in radiation injuries of the dermis, the obliterative endarteritic change in the blood vessels is always accompanied by elastotic degeneration of the vessel wall itself.

Yet another reason for our intense interest in elastotic degeneration of connective tissue fibers, especially in the skin, is the finding, described fully elsewhere, of the almost consistent epidermal changes associated with elastotic degeneration of the underlying dermis. Our experiments in normal human volunteers strongly suggest that the presence of abnormal exogenous collagen may provoke profound epidermal hyperplasia and invasiveness in healing injuries of the skin. Resolution of this epidermal hyperplasia induced in healing Thiersch-graft donor sites by treatment with homodermal grafts is followed, weeks later, by the appearance in these same areas of small or large plaques of elastotic degeneration of previously healthy dermal connective tissue. The sec-

ondary and spontaneously occurring elastotically degenerated dermal plaques seem, invariably, to evoke epidermal hyperplasia and invasiveness localized to the zone of elastotic degeneration. Furthermore, the consistent association of elastotic degeneration of the dermis with precancerous and cancerous epidermal lesions, attributable to irradiation injuries, coupled with our findings in healthy, nonirradiated human volunteers, suggests that elastotic degeneration, produced as a result of endogenous metabolic dyscrasias, may also be of considerable significance in the pathogenesis of carcinoma, at least in the skin.[44]

It seems to be of primary importance to define chemically the nature of these changes in the connective tissues, more especially since Orr,[30] Marchant and Orr,[45] Vernoni,[22] Jolles and Koller,[46] and Jolles [47] have previously provided strong evidence to suggest that alterations in dermal connective tissue may play an important part in the pathogenesis of skin cancers. Moreover, Vernoni has even suggested that elastic fibers, or derivatives thereof, may have growth-promoting properties.*

It may also be possible that a local liberation of hyaluronic acid from degenerating connective tissue fibers or ground substance may occur in association with elastotic degeneration and that this hyaluronic acid may produce local scarring or epidermal hyperplasia and "invasiveness" such as that described by Shifrin and co-workers [49] and later by Simpson.† It also seems possible, from the writing of many investigators, including Lansing, Rinehart and Greenberg, Gersh and Catchpole,[50] Gross,[27] and Bunting and Bunting;[51] from the remarks of many contributors to the Macy Foundation Conferences on connective tissues, and from our own work, that a full understanding of these fibrillar degenerations may shed new light on our appreciation of several degeneratory diseases and of many of the phenomena of ageing in man.

Until precise physical and chemical data concerning connective tissue fibers become available, inaccuracies, misunderstandings, and much confusion presently prevailing in this field could be diminished by characterizing fibers on the basis of the more detailed and precise tinctorial criteria enumerated above.

Our findings also indicate that the generally accredited standard histological methods for demonstrating elastic fibers cannot be relied on to stain only "normal" elastic fibers. The views expressed by Hall,[11] Banga,[10] and Gross [27] concerning the difficulties in distinguishing between elastic, collagen, and other connective tissue fibers, using chemical and submicroscopic morphological criteria, can be substantiated on the basis of our own findings, using less elegant and less precise histological methods.

We are thoroughly aware of the limitations of staining methods in defining chemical changes in tissues, but nevertheless we firmly believe that careful observations, such as those recorded here, often form the starting point for investigations which will ultimately lead to the clarification of fiber structure, by the use of the most modern histochemical, chemical, and biophysical methods and criteria, most of which are not available to our group. Some indication of what may be achieved if attempts are made to explain the tinctorial reactions, used widely, effectively, and for many years by histologists and histopathologists, is available from a recent outstanding publication by Glegg, Eidinger, and Leblond.[52] By the combined use of histological, chemical, and physical methods, these investigators were able to provide a satisfactory explanation for some of the differences in staining properties of reticular and collagenous fibers.

We sincerely hope that the precise nature of the above-mentioned tinctorially definable alterations in connective tissue fibers in dermis, blood vessels, and gall bladder will also soon be elucidated in terms of the chemistry and ultrastructure of connective tissue fibers.

* References 22 and 48.

† Simpson, W. L.: Connective Tissue and Cancer, in Asboe-Hansen,[3] p. 225.

SUMMARY AND CONCLUSIONS

Attention is redirected to the occurrence in blood vessels, skin, and gall bladder of so-called "increases in elastic fibers" in certain disease states.

On the basis of morphological and tinctorial reactions it has been found possible to distinguish between "normal" elastic fibers and abnormal "pseudoelastic" fibers.

The name "elastotic degeneration" of collagen fibers is suggested for these abnormal fibers which, while they stain with all recognized elastic fiber stains, nevertheless fail to stain with other dyes which seem to delineate normal elastic "membranes." These elastotically degenerated fibers may also stain with dye mixtures which do not stain "normal" elastic membranes.

It is shown that not all fibers which stain with accepted and routinely used stains for elastic fibers can be accepted as unaltered elastic fibers.

The suggestion is made that elastotically degenerated fibers are formed either by a degeneration of preexisting collagen or, *de novo*, as a result of disturbances in the metabolic processes normally leading to collagen or reticulum fiber deposition in connective tissues.

It is also suggested that elastotic degeneration of collagen is a precursor of calcification of blood vessels and gall bladder and may play some role in the pathogenesis of skin carcinoma.

A grant from the Schlesinger Organization made the execution and the presentation of this work possible. Mrs. Florence Powell made a grant-in-aid of a cancer research library. Mrs. B. H. Robinow gave assistance in obtaining journals. Mr. R. Stuart prepared the prints of the photomicrographs. Mrs. H. Herzog assisted throughout the investigation.

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Books

Pediatric Diagnosis. By Morris Green, M.D., and Julius B. Richmond, M.D. Price, $10.00. Pp. 436. W. B. Saunders Company, 218 Washington Sq., Philadelphia 5.

This book represents an effort to present the diagnostic aspects of pediatrics from the standpoint of differential diagnosis of patient complaints rather than by means of the conventional organ system disease tabulation. The book approaches the patient as the physician does, by the sequence of history, symptoms and signs, and differential diagnosis. Thus, fever as a symptom lists and briefly discusses the cause of fever, and cyanosis as a finding leads to discussion of the pathogenesis and etiology of this physical sign. The differential diagnosis of most symptoms and signs are discussed in this manner.

The book opens with an excellent section on the pediatric history and closes with an unusual section on health supervision. Throughout the book appropriate modern references are listed with the subject under discussion, a reasonable and logical place for references.

The book was read with considerable pleasure. While designed for quick reference and with no pretense of being encyclopedic, the volume is stimulating in its fresh approach to bedside medicine. The book accomplishes its purpose of accenting competence in history taking and physical examination and encourages the application of basic science in formation to clinical situations and differential diagnosis. It should prove popular as an auxiliary text and quick reference.

The Ciba Foundation Symposium on the Kidney. Edited by A. A. G. Lewis and G. E. W. Wolstenholme. Price, $6.00. Pp. 333, with 125 illustrations. Little, Brown & Company, 34 Beacon St., Boston 6, 1954.

This volume is a verbatim account of the proceedings at the International Symposium on the Kidney, arranged by the Ciba Foundation and the Renal Association. It was held July 7-10, 1953, in London. The thirty-five participants were chosen for their outstanding contributions in those aspects of renal anatomy, physiology, pathology, and medicine discussed, and included experts from Belgium, Denmark, England, France, Holland, Sweden, Switzerland, and the United States. Twenty-two papers were read and discussed. These covered structural and functional relationships in the kidney, the regulation of acid base balance as well as other tubular functions, general problems of electrolyte excretion, and the renal role in the volume content of the body fluid.

The book is readable, compact, and adequately illustrated. References are included for each presentation. It will be useful to all interested in the kidney and its disorders, although much of its content will be familiar to those actively following the current literature in this field.

Emergency Treatment and Management. By Thomas Flint, Jr., M.D. Price, $5.75. Pp. 303. W. B. Saunders Company, 218 Washington Sq., Philadelphia 5; W. B. Saunders Company, Ltd., 7 Grape St., Shaftesbury Ave., London, W.C. 2, 1954.

Every Emergency Room should have a small, concise yet complete reference book. *Emergency Treatment and Management,* by Dr. Thomas Flint, Jr., is a partially successful attempt to satisfy that need. The book is set up in outline form. The headings list complaints and specific diseases, followed by symptoms, treatment, and prognosis.

The author far too often suggests that narcotics be given on an emergency basis and also recommends that antibiotics be used on an emergency basis for nonspecific diseases, such as bursitis. It was disturbing to read that the suggested treatment for Colles' fracture was plaster immobilization after reduction from axilla to the proximal interphalangeal joints. Such immobilization frequently leads to stiffening of the metacarpophalangeal joints.

Over one-third of the book is concerned with poisons. Again in brief outline form, symptoms are listed and treatment recommended for intoxication by drugs from acetanilid to Zygadenus. An appendix to this chapter of commercial preparations and their principal toxic ingredients should be very useful.

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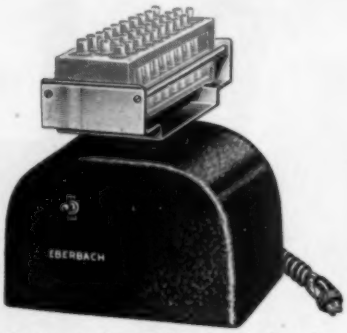

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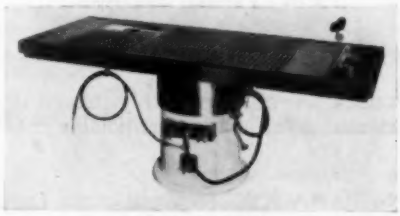

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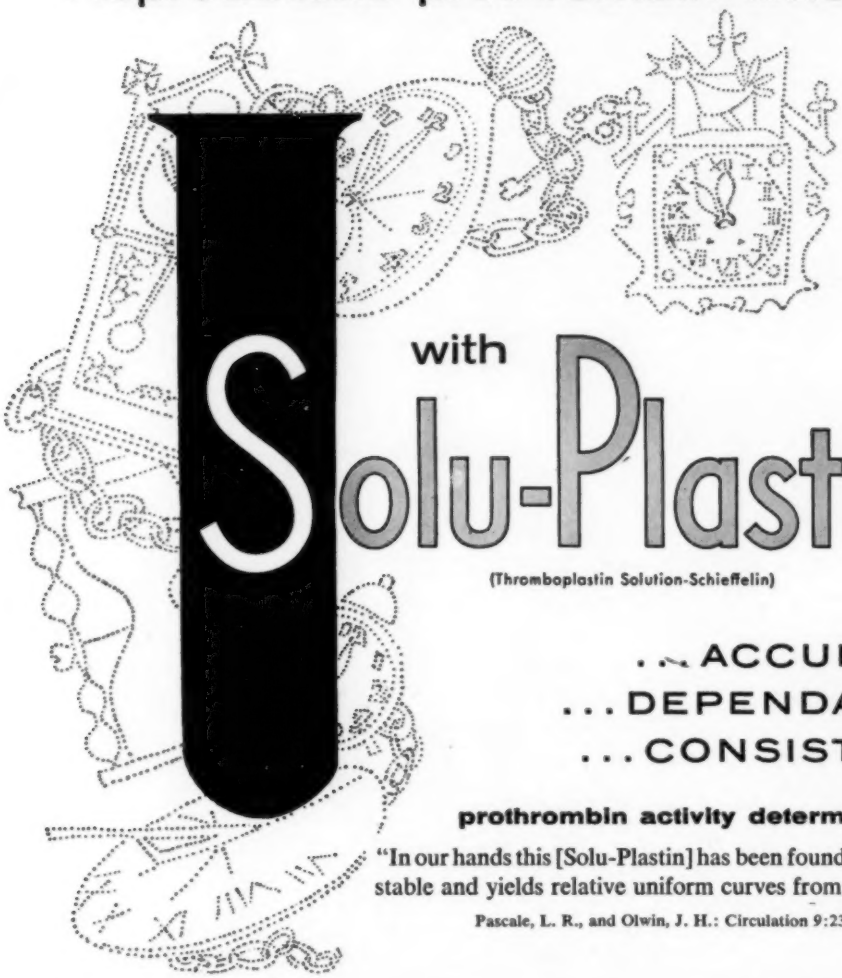

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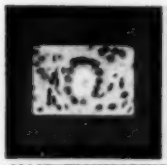

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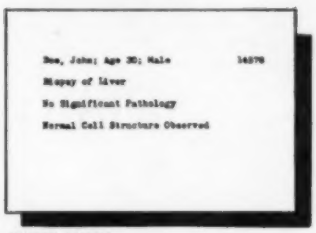

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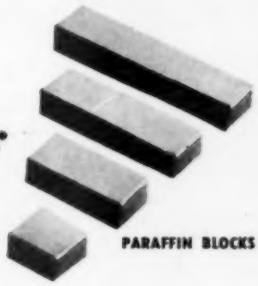

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